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chromatography of alkaloids

*part B: gas-liquid chromatography and
high-performance liquid chromatography*

*R. Verpoorte and
A. Baerheim Svendsen*

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GAS—LIQUID CHROMATOGRAPHY

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PREFACE

Most naturally occurring alkaloids are fairly high-molecular-weight compounds. As such they are usually only slightly volatile or non-volatile. The application of gas chromatography to the analysis of alkaloids is, therefore, limited.

When working with compounds of high molecular weight and low volatility in gas chromatography, it is often necessary to increase their volatility or to reduce their polarity by converting them to special derivatives. Usually it is also necessary to work with gas chromatographic column with a low percentage of stationary phase, in order to be able to operate at relatively moderate column temperatures¹.

The injection of an alkaloid or a mixture of alkaloids into the injection port of the gas chromatograph may be of vital importance for the further course of the analysis. One has to pay attention to the fact that the substance to be analyzed may be in many cases degraded during the analysis due to catalytic effects of the metal parts of the gas chromatograph, especially to the injection port, where the temperature has to be relatively high to obtain an instant and complete evaporation of the compounds.

The high temperature of the injection port required for the evaporation of the alkaloids may sometimes lead to decomposition, such as dehydration, hydrolysis or transesterification. Atropine can under certain conditions be dehydrated to apoatropine. The degree of dehydration has been found to be associated with the amount of glass wool on the top of the column material. Diacetylmorphine is eluted as a sharp well-defined peak when chromatographed alone. In mixtures with codeine, morphine or other phenolic or alcoholic substances transesterifications take place in the injection port, giving rise to several new esters not present in the original solution. 6-O-Acetylmorphine gives peaks of morphine, 6-O-acetylmorphine and diacetylmorphine. 3-O-Acetylmorphine is more stable and may be gas chromatographed with little or no decomposition².

To prevent an undesirable degradation of the compounds to be analyzed, glass columns have mostly been used for gas chromatography of alkaloids because they are indifferent to the compounds. Possible catalytic decomposition of sensitive compounds and adsorption phenomena caused by metal columns, e.g. copper, aluminium and stainless steel, may, however, be eliminated in some cases by a simple coating of the tubing material with the stationary phase used in the analysis. The decomposition and adsorption phenomena disappear^{3,4}. Codeine and noscapine³ and ephedrine⁴ were successfully gas chromatographed on coated, packed metal columns without decomposition or adsorption.

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I. GENERAL PART

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Chapter 1

PACKED COLUMNS

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A successful gas chromatography depends to a great extent upon the quality of the columns used. For packed columns Roman et al.¹ showed that the quality of such columns is proportional to the care bestowed upon the preparation procedure. In comparing some commercially available solid supports the authors found that poor gas chromatographic performance was often caused by adsorptive sites on the surface of the solid support, *e.g.* by incomplete deactivation of the support. However, the quality could be improved by more careful, eventually repeated, deactivation of the support.

Most authors prefer to make their columns themselves. In the literature a number of recommendations for preparation of packed columns for high boiling, slightly volatile compounds are given. Most of them are based on empirical observations, made when performing gas chromatography to solve specific analytical problems. When satisfactory results were achieved, no need for further evaluation of the column was found to be necessary, and the procedure used was therefore assumed to be generally applicable. When adopted by others, they became gradually transformed into some kind of magic!

For gas chromatography of alkaloids low load packed columns are usually used. In such cases "tailing", caused by active adsorptive sites, is often observed.

With gas-liquid chromatography a separation is achieved by the differences in the partition coefficients of the various compounds between the gaseous mobile phase and the stationary liquid phase. However, in gas-liquid chromatography with low load packed columns, the gas-liquid partition equilibrium is influenced by the properties of the solid support, because of active adsorptive sites on the surface of the support, and inhomogeneous coating of the support, which leaves parts of the support uncoated. In both cases adsorption of the solutes to the support material can take place. The effect is especially troublesome with small sample sizes. Various techniques have been developed to deactivate the active sites on the solid support as well as to assure a homogeneous coating. The deactivation has mostly been achieved by acid and alkaline washing, often followed by chemical deactivation, lastly by precoating of it with a very small amount of a polar stationary phase.

1.1. Deactivation of solid support

1.1.1. Deactivation of solid support by acid and alkaline washing

The methods which have mostly been used to deactivate the active adsorptive sites on solid supports of diatomaceous earth type include acid washing and alkaline washing. Acid washing of such support material was recommended by James and Martin². They suspected metallic oxides to be at least partly responsible for the adsorptive properties. Washing with concentrated hydrochloric acid followed by rinsing with water until neutral, was intended to remove these metallic oxides. Several other authors have clearly shown that the acid washing is an important step prior to silanization.

In some cases an alkaline washing has been applied in addition to the acid one, as a pre-treatment for silanization. The combination was thought to be more effective for removing amphoteric ions, such as aluminium, than acid washing alone. Holmes and Stack³ prepared packing material with low adsorptive properties in this way, so also Brochmann-Hanssen and Baerheim Svendsen for their analysis of barbiturates, sympathomimetic amines and alkaloids⁴⁻⁶.

Comparative studies to investigate the influence of acid and alkaline washing on a solid support, such as Chromosorb W, prior to silanization and coating with the stationary phase by a) acid washing, b) acid washing followed by alkaline washing and c) acid washing followed by alkaline washing and then again acid washing, were carried out by Meilink⁷. He recommended the following procedure to achieve optimum deactivation:

Suspend Chromosorb W in hydrochloric acid (25 %), filter off after two days by suction and rinse the Chromosorb with distilled water until all acid is removed. Remove most of the water by washing with methanol, filter and dry the product in a rotary evaporator under reduced pressure at 90°C. Suspend the acid washed material in 1 mole per liter KOH in methanol. Filter off after 15 hours and rinse with methanol until free of KOH. Dry as described above. Treat the acid and alkali washed material once more with hydrochloric acid as described above, rinse it with water and dry it as described above.

1.1.2. Deactivation of solid support by chemical deactivation

An acid and base washing of the solid support is usually followed by a chemical deactivation - mostly in the form of silanization. Horning et al.⁸ used gaseous dimethyl dichlorosilane (DMDCS) for this purpose on the acid washed support. Horning et al.⁹ emphasized that acid washing was essential. Bohemen et al.¹⁰ pointed out the risk that DMDCS silanization might leave active chlorine groups behind, which in their turn could be converted to hydroxyl groups when brought into contact with water. This problem will not be encountered if hexamethyldisilazane (HMDS) is used as silanizing reagent.

According to Bohemen et al.¹⁰ acid prewashing is not necessary for HMDS silanization. Brochmann-Hanssen and Baerheim Svendsen^{4,5,6} obtained well deactivated support on treatment with HMDS; neither acid nor base washing were, however, omitted. Sawyer and Barr¹¹ found HMDS silanization very effective for deactivation purposes, although a slight residual adsorptive activity was left.

Compared with DMDCS silanization, a disadvantage of HMDS should be mentioned. McMartin and Street¹² found that a support which had been well deactivated by HMDS was reactivated at

temperatures above 260°C.

Alkaline treatment of the solid support directly prior to silanization with DMDCS seems not to be advantageous. The presence of free hydroxyl groups on the support, obtained by acid washings, seems to be essential for the silanization reaction with DMDCS. According to McMartin and Street¹² the acid washing can be omitted when DMDCS silanization is performed on "wet" or "damp" solid support. They assumed that hydrochloric acid, released in the reaction of DMDCS with water, replaces the acid washing. They also supposed that polymerization of DMDCS takes place in the presence of water, resulting in a chemically bonded polysiloxane layer on the support. If higher concentrations of water in the support than about 5 % are used, excessive formation of gaseous hydrochloric acid will be the result.

Meilink⁷ preferred the procedure involving acid, base and acid washing to obtain a best possible deactivation of the support.

Recommended procedure for silanization with DMDCS:

Suspend the acid, base and again acid washed solid support in DMDCS in toluene (5 % v/v), treat the suspension in an ultrasonic bath for 5 minutes and filter off the solvent after 24 hours. Wash with dried toluene and dried methanol under exclusion of water and dry the support material in a rotary evaporator under reduced pressure at 90°C.

Bohemen et al.¹⁰ recommended the following procedure for silanization with HMDS:

Dry the support under vacuum at 150°C. Cover 25 g of this sample, while still warm, with a mixture of 80 ml of light petroleum (b.p. 60-80°C) and 15 ml hexamethyldisilazane. Heat the mixture on a steam-bath and reflux for 1 hr. Use a drying tube of calcium sulphate at the condenser exit. After refluxing, add 2 ml of *n*-propanol; this helps materially by wetting the support, and although it reacts with unchanged hexamethyldisilazane to form SiMe_3OPr , this in turn reacts with hydroxyl groups in the same way as the parent silazane. After 30 hr. heat the mixture again and reflux for several hours. Wash the support with light petroleum (2 x 50 ml), then *n*-propanol (1 x 50 ml), and then again with light petroleum (2 x 50 ml). Finally, filter off the support and dry it for 2 hr. on a steam-bath in an atmosphere of nitrogen.

1.1.3. Deactivation of solid support by chemical bonding of stationary phase

Active groups on the support can also react with other than silanizing agents. In that way small amounts of stationary phases can be chemically bonded to the solid support as esters of the (-Si-O-R) type. According to Grushka and Kikta¹³ such esters are liable to hydrolysis. Chemical bonds of the (-Si-O-Si-R) type, arising from silanization with organosilanes, are more stable. The main advantage of gas chromatographic packings with chemically bonded stationary phases, compared to physically coated ones, is their greater thermal stability. The upper temperature limit lies about 80-90° above that of the corresponding nonbonded ones¹⁴.

Two types of reaction procedures have been described. In the first one the coupling reaction is brought about at an elevated temperature. Aue et al.¹⁵ stated that polyethylene glycol 20M (PEG 20M) could not be fully washed off a support with methanol and methylene chloride after heating to 280°C. The remaining PEG 20M layer was too thin to be measured with analytical combustion techniques. Hastings and Aue¹⁶ demonstrated that chemical bonding of PEG to support material leads to well deactivated products. These "polymer deactivated" products proved to be good supports when they were physically coated with conventional station-

ary phases. The stationary phase that was used, then determined the characteristics of the packing, and not the ultra-thin, non-extractable PEG-film. Moseman¹⁷ and Winterlin and Moseman¹⁸ used PEG deactivated supports coated with QV-210 for gas chromatography of pesticides. They stated that the PEG deactivated support was superior to non-deactivated materials; no comparison was, however, made with silanized supports. The method of chemical bonding was modified by Daniewski and Aue¹⁹. They refluxed the support in a solution of PEG instead of dry heating the PEG coated support.

The coupling reaction can also be realized by using a chlorosilane as an intermediate to "activate" the support surface. Chlorosilanes react easily with the active sites on the support. When trichloromethylsilane is used, always at least one active chlorine group is left, which in its turn can react with alcohols, such as polyethylene glycols. In this way, Mori¹⁴ prepared Chromosorb W (AW) with chemically bonded PEG, resulting in a loading of 4.2 % with PEG 20M and of 2.0 % with PEG 3000.

Both thermally and chlorosilane-mediated PEG-bonded supports can be used as gas chromatographic packings without further coating^{14,17}.

Because the chemically bonded PEG molecules are thought to be arranged on the support surface like "bristles of a brush", Mori¹⁴ concluded that the rate of mass transfer should be increased, making chemically bonded stationary phases very suitable for gas chromatographic separations.

Recently Street et al.²⁰ acylated diatomaceous earth with benzoyl chloride in pyridine as a pretreatment for coating. They reported that a marked reduction in adsorption could be obtained, enabling polar compounds, such as morphine, to be gas chromatographed in nanogram amounts without derivatization. A 1000-fold improvement in chromatographic capability could be obtained, compared to the best conventional commercial packing.

Recommended procedure for thermal bonding of PEG 20M:

Coat acid-base-acid washed Chromosorb W with 5 % (w/w) PEG 20M using the filtration technique of Horning et al.⁸ and fill a glass gas chromatographic column with the material. Flush the column with nitrogen (60 ml/min) for 6 hours and heat at 280°C for 15 hours with a nitrogen flow of 3.5 ml/min. Empty the column and rinse the packing material thoroughly with methanol and methylene chloride respectively, followed by extraction with methylene chloride for 6 hours in a Soxhlet apparatus. Dry the support in a rotary evaporator at 60°C and coat it with the stationary phase that was chosen.

Recommended procedure for chlorosilane-mediated bonding of PEG 4000:

Suspend 18 g acid-base-acid washed Chromosorb W in a mixture of 50 ml dried toluene and 25 ml tetrachlorosilane (TCS), treat it for 5 min. in an ultrasonic bath to remove air, filter off the solution after 4 hours at room temperature, rinse with dried toluene under exclusion of water to remove the TCS completely. While it is still excluded from atmospheric moisture, suspend the TCS treated support in a solution of 8 g PEG 4000 in 75 ml dried toluene for 48 hours at 50°C. Filter the PEG solution off and rinse the mass with toluene and methylene chloride successively, followed by extraction with methylene chloride in a Soxhlet apparatus. Dry the mass in a rotary evaporator at 60°C.

1.1.4. Deactivation of solid support by precoating with small amounts of a polar stationary phase

To diminish any eventual residual adsorptive activity of the support that may still be present after deactivation by acid and alkaline washing, and by chemical procedures, Bohemen et al.¹⁰ introduced a precoating with 0.1 % PEG 400. The PEG molecules are thought to be adsorbed tightly to the residual active sites of the support. This precoating procedure was also used by Brochmann-Hanssen and Baerheim Svendsen⁶ in their gas chromatographic studies on alkaloids. They preferred to apply PEG 9000 0.1 % (w/w). The higher molecular weight was used as higher temperatures were employed. Kabot and Ettre²¹ recommended to apply the main stationary phase by means of a solvent "in which the precoated polar phase (PEG) is insoluble". Lipsky and Landowne²² dissolved both the polar phase (PEG) and the non polar phase in the same solvent and carried out both precoating and coating simultaneously. They found that this procedure reduced or eliminated tailing due to non-linear sorption isotherms, and attributed the effect to deactivation of the support.

The bonding of PEG to the active adsorptive sites on the support can take place in two different ways. First, some chemical bonding may occur when the packing is used at higher temperatures. This effect is comparable with the thermal bonding of PEG as described by Aue et al.¹⁵. On the other hand, strong hydrogen bonding may be involved, as stated by Evans et al.²³. They used amine antioxidants as deactivators. These substances may be expected to combat oxidative degradation of the stationary phase in addition to their deactivating properties. They suspended the support, which had not been deactivated by any previous silanization, in a solution of the compounds. The solution was suctioned off, but the amine antioxidant molecules remained bonded to the active sites of the support. The support was then coated with a stationary phase dissolved in a solvent, which did not displace the amine antioxidant from the support. In that way the amine antioxidant served as a deactivator between the stationary phase and the support.

1.2. Coating of solid support with stationary phase

Since Horning et al.⁸ gas chromatographed several kinds of compounds on solid support thinly coated with a stationary phase, the use of such packing material has become increasingly common for gas chromatography of high boiling, slightly volatile compounds. The reason is obvious: The retention times are distinctly shortened, facilitating gas chromatography of such compounds at relatively low column temperatures. However, with a decrease in the percentage of stationary phase, the risk of getting uncoated areas on the surface of the support increases. According to Bohemen et al.¹⁰ a support with less than 5 % of stationary phase greatly enhances the influence of the solid support on the gas chromatographic performance.

A frequently used coating procedure for packing materials with a low percentage of stationary phase was described by Horning et al.⁸. It is generally called the "filtration technique". After the solid support has been suspended in a solution of the stationary phase, enough of the solution is filtered off to ensure that the support on drying will contain the desired amount of stationary phase. Because only small amounts of the solution are left after filtration, great differences in the concentration of the stationary phase in the moist mass during the evaporation will not occur.

McMartin and Street¹² used another technique to obtain the same result: They rinsed the

support with a solution of the stationary phase and dried it on a hot plate, while stirring gently with a glass rod. However, they did not indicate how a definite percentage of stationary phase on the support could be obtained. Parcher and Urone²⁴ prepared packing material by "solution coating" and fluidized drying. The percentage of stationary phase on the support depended on the concentration of the solution used. Averill²⁵ coated solid supports, when packed in gas chromatographic columns, by passing a solution of the stationary phase through the column. Although reproducible results were obtained when the coating was carried out in exactly the same manner, no details of the percentage of stationary phase on the support were given.

Recommended coating procedure:

Suspend acid washed, silanized solid support (Chromosorb W) in a solution of the stationary phase in a suitable solvent to obtain the desired percentage of stationary phase after evaporation of the solvent. Treat the suspension for 5 minutes in an ultrasonic bath to remove air from the support and evaporate the solvent in a rotary evaporator at the boiling point of the solvent until particles of the support begin to stick together. Stir the mass gently with a glass rod under continuous heating in a current of air. Care must be taken not to damage the particles of the support. Continue evaporation in this way until the mass has got flowing properties. Continue the evaporation in the rotary evaporator, and raise the temperature gradually to about 100°C and heat at that temperature for 30 minutes.

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Chapter 2

CAPILLARY COLUMNS

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2.1. CAPILLARY COLUMNS

Since Desty et al.¹ introduced glass capillary columns in the gas chromatographic analysis of petroleum products many scientist have been involved in developing procedures to prepare high quality glass capillary columns. This was achieved by leaching conventional glass, followed by high temperature silylation.

Since most chromatographic separations of alkaloids on capillary columns employ temperature programming and often relatively high temperatures, the stability of a coated column at higher temperatures is of the utmost importance. Depolymerization of the stationary phase can take place because of impurities in the stationary phase itself, or in the glass wall (metal salts, silanol groups, straight siloxane bridges) or in the actual silica surface structure.

Chemical bonding of stationary phases has been shown to increase the stability of the stationary phase compared with conventionally coated films. A chemically bonded phase may be regarded as one that is not extractable by solvents that do not attack the phase.

Conventional columns can be basic, they can be made in any internal diameter, they can be whiskered, and they can, therefore, be coated effectively with any phase.

Fused silica columns have obvious advantages, but do not have these possibilities. The fused silica capillary columns do not solve all the problems. The activity of silicium based capillary columns (glass or fused silica) is due to the trace of metal ions, silanol groups and siloxane bridges. Whereas a leached soda-lime column is always basic, fused silica columns are always acidic.

It may, therefore, be concluded that fused silica and leached glass columns coated with polysiloxanes (OV-1, OV-101, OV-73 and SE-54) and polyethylene glycols (Superox 20M) provide a good choice for capillary gas chromatography of alkaloids. The quality of fused silica and leached glass columns is practically equal². In a number of papers the preparation of glass capillaries for the analysis of alkaloids has been described, as well as the inactivation of such glass capillaries, and the chemical bonding of stationary phases on glass capillaries^{3, 4, 5, 6}.

The injection of the sample of slightly volatile compounds, such as alkaloids, in capillary gas chromatography can be carried out in different ways depending upon the kind of column used. Verzele et al.² stated that the "cold on column injection" and the "falling needle injection" were the best alternatives for quantitative gas chromatography since they were applicable to high temperature capillary gas chromatography. The sampling system in capillary gas chromatography has been dealt with in a series of papers^{7, 8, 9}.

Although the high temperature stability of many capillary columns with non extractable stationary phases has eliminated the need for derivatization of many compounds, derivatiza-

tion can often improve the specificity and the sensitivity. Derivatization can be carried out - especially by converting polar compounds into non-polar compounds - before the injection of the sample, or by means of flash heater derivatization¹⁰. Derivatization may in many cases increase the resolution and the differences in retention time of a compound, and the derivative may give extra information during the identification of unknown compounds. On-column derivatization in gas chromatography has been described in a number of papers^{10,11, 12,13}.

Capillary columns for the gas chromatography of alkaloids were first described by Massingill and Hodgkins¹⁴. They investigated a number of alkaloids on three different stationary phases: Apiezon L, SE-30 and QF-1:

Apiezon L 100 feet by 0.01 inch I.D., temperature programming 100-200°C, 12°C/min

SE-30 200 feet by 0.01 inch I.D., temperature programming 100-250°C, 12°C/min

QF-1 100 feet by 0.01 inch I.D., temperature programming 100-250°C, 12°C/min

The results are shown in Table 2.1.

TABLE 2.1

RETENTION DATA OF ALKALOIDS ON CAPILLARY COLUMNS¹⁴

M.W. = Molecular Weight, t_R = retention time, $t_{R(rel)}$ = relative retention time, Nic = nicotine, N.R. = no response, ft = feet

Alkaloid	M.W.	100 ft QF-1		200 ft SE-30		100 ft Apiezon L	
		t_R (min)	$t_{R(rel)}$ (Nic = 1)	t_R (min)	$t_{R(rel)}$ (Nic = 1)	t_R (min)	$t_{R(rel)}$ (Nic = 1)
Tropinone	139	1.58	0.79	3.42	0.82	1.33	0.40
Tropine	141	1.33	0.67	3.17	0.76	1.66	0.50
Nicotine	162	2.00	1.00	4.17	1.00	3.33	1.00
Anabasine	162	4.08	2.04			10.08	3.03
(Neonicotine)							
Ephedrine	165	2.33	1.17	5.25	1.25	6.00	1.80
Gramine	174	2.42	1.21	6.83	1.64	4.00	1.20
Caffeine	194	8.17	4.09	11.58	2.77	N.R.	
Bufotenine	204	11.50	5.75				
Pilocarpine	208			22.42	5.40		
Isopilocarpine	208						
Procaine	236	11.42	5.71	15.00	3.60		
Homatropine	275	9.08	4.54	11.75	2.82		
Atropine	289	7.58	3.79				
Piperine	285			18.33	4.40		
Cinchonidine	294			18.58	4.46		
Cinchonine	294						
Scopolamine	303	9.75	4.88	16.08	3.85		
Ajmaline	326	N.R.		N.R.			
Strychnine	334	N.R.		N.R.			
Papaverine	339	N.R.		N.R.			
Aspidospermine	352	N.R.		N.R.			
Berberine	353	N.R.		N.R.			
Conessine	356	N.R.		N.R.			
Brucine	394	N.R.		N.R.			
Colchicine	399	N.R.		N.R.			

The Apiezon L capillary column was useful only for alkaloids with molecular weights about 175. At 125°C (isothermal) it separated nicotine and anabasine by six minutes, but tailing was pronounced. The SE-30 capillary column resolved the lower molecular weight (< 200) al-

kaloids very well, but the higher molecular weight (~ 300) alkaloids were not so well resolved. Tailing was apparent for most of the samples, but was not pronounced. The QF-1 capillary column gave good resolution of tropine-tropinone, nicotine-anabasine, and atropine-homatropine-scopolamine mixtures. Generally the peaks were extremely sharp with practically no tailing. The QF-1 capillary column out-performed the other capillary columns and gave good resolution of alkaloids with molecular weights up to 303. However, a comparison of the separation of atropine-homatropine-scopolamine on a 6 feet by 1/8 inch 1 % packed QF-1 column with that of the 100 feet QF-1 capillary column showed that the packed column gave better resolution.

Since the first paper of Massingill and Hodgkins¹⁴ appeared, the use of capillary columns in the analysis of alkaloids has been limited. A few examples of applications are mentioned below.

Harke and Drews¹⁵ used a 50 m long stainless steel capillary column, 0.5 mm I.D., coated with Ucon LB 550 X (polyethylene glycol) and KOH for the separation of tobacco alkaloids. A typical chromatogram of a test-mixture containing 3-pyridyl-*n*-propylketon, nicotine, nor-nicotine, myosmine, anabasine and nicotyrine is shown in Figure 5.1 (Tobacco alkaloids).

To identify tobacco alkaloids and their mammalian metabolites, Pilotti et al.¹⁶ made use of capillary gas chromatography-mass spectrometry using glass capillary columns (33 m by 0.4 mm I.D.) coated with Emulphor-O, or a capillary (9.6 m by 0.2 mm I.D.) coated with OV-101. The gas chromatographic data are summarized in Table 5.10 (Tobacco alkaloids).

Bohn et al.¹⁷ used glass capillary gas chromatography in investigations of illicit heroin samples and obtained good separation of heroin, 6-O-monoacetylmorphine, acetylcodeine and caffeine on a 12 m by 0.3 mm I.D. glass capillary coated with Triton X 303 (Merck) and temperature programming from 200°C to 250°C.

Dow and Hall¹⁸ used a combination of capillary gas chromatography and mass spectrometry to estimate nicotine in plasma by selective ion monitoring. The capillary was 20 m by 0.3 mm I.D., coated with SP 1000 and the temperature 160°C.

For capillary gas chromatography of alkaloids and other high boiling compounds stainless steel or borosilicate glass capillaries have mostly been used. Verzele et al.², in a study on high temperature quantitative glass capillary chromatographic analysis of piperine and quinine-quinidine, found that untreated soft glass gave better results than borosilicate glass. Some occasional tailing could be removed by the analysis of quinine-quinidine by sodium chloride dendrite deposition. With OV-1, OV-17, OV-225, Superox-4, RSL-702 and RSL-903 good peaks were obtained. The resolution of quinine and quinidine was zero on OV-1, but improved with increasing polarity of the stationary phase and was complete only on RSL-903 (a highly polar polyaromatic sulfone), the most polar phase of the series. A 30 m by 0.3 mm I.D. sodium dendrite column coated with 0.15 μ m layer of RSL-903 was used. A moving needle injector proved to be the best choice in order to obtain accurate quantitative results. One μ l of the solution to be analyzed was introduced on the needle point and the sample injected isothermally at 280°C. Figure 2.1 and 2.2. show chromatograms of some pure *Cinchona* alkaloids and of such alkaloids in a *Cinchona* bark pharmaceutical preparation. For the quantitative assay, piperine, eluting shortly after quinine, was used as an internal standard.

The isothermal analysis was applied to the assay of quinine in soft drinks, and of quinine and quinidine in pharmaceutical preparations, with good results. By multiple analysis the standard deviation for quinine in soft drinks was found to be 1.97 %, and for quinine-quinidine

FIGURE 2.1

GLASS CAPILLARY GAS CHROMATOGRAPHY OF QUININE AND RELATED ALKALOIDS²

on a 30 m by 0.3 mm I.D. soft glass capillary with sodium dendrite deposition and RSL-903 as stationary phase; temperature 280°C; falling needle injection. 1 = cinchonine, 2 = cinchonidine, 3 = quinidine and 4 = quinine.

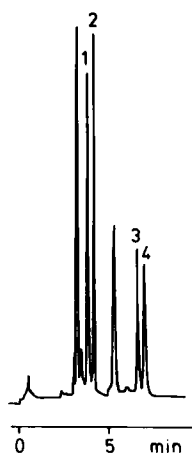
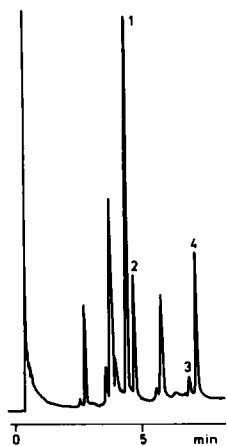


FIGURE 2.2

GLASS CAPILLARY GAS CHROMATOGRAPHY OF ALKALOIDS FROM A *CINCHONA* BARK PHARMACEUTICAL PREPARATION².

GLC conditions and peak numbers as given in Figure 2.1. Unnumbered peaks unidentified.



dine in pharmaceutical preparations 1.07 % and 0.90 % respectively.

For the assay of piperine in pepper, a 25 m by 0.5 mm I.D. glass capillary deactivated by high temperature silanization and coated with OV-1 was used. The samples were injected by the on-column injection technique at 100°C, as described by Grob and Grob Jr.¹⁹ The standard deviation of the whole procedure, sample preparation and chromatographic analysis, was 2.5 %.

Verzele et al.² concluded that the "cold on-column" injection, applied for the piperine assay, and the "falling needle" injection, applied for the quinine-quinidine assay, are the best alternative at the moment for quantitative capillary gas chromatography. They are applicable to high temperature capillary gas chromatography, but they also have their limitations.

Floberg et al.²⁰ applied glass capillary gas chromatography for the analysis of theophylline and caffeine in plasma. The alkaloids were analyzed as such, or after derivatization. Severson et al.²¹ also used glass capillaries for the separation and quantification of tobacco alkaloids, whereas Edlund²² separated morphine, 6-O-monoacetylmorphine and codeine from plasma samples after derivatization. He stated, however, that degradation will always occur during gas chromatography on the columns used, since no absolutely inert column is available. In the cases studied by him, the degradation was very reproducible, so that quantitative analysis could be carried out in spite of degradation. Gas chromatography on glass capillaries was also applied by Neumann und Gloger²³ in "fingerprinting" illicit heroin samples directly, and after derivatization.

The development of the cold on-column injection technique and fused silica column with non-extractable stationary phases opened new ways in the analysis of underivatized drugs, such as alkaloids. Plotczyk²⁴ applied the cold on-column injection technique and fused silica capillary columns for the analysis of *i.a.* cocaine, codeine and quinine. Demedts et al.²⁵ introduced fused silica capillary columns in the toxicological analysis of illicit heroin samples. With a NP-detector and permanent deactivation of the column with polysiloxane, excellent results were obtained for heroin, down to the low nanogram range, without derivatization.

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Chapter 3

DERIVATIZATION OF ALKALOIDS FOR GAS CHROMATOGRAPHY

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3.1. DERIVATIZATION OF ALKALOIDS FOR GAS CHROMATOGRAPHY

Owing to the low volatility and thermal instability of many alkaloids, derivatization is performed to give them better gas chromatographic properties. Derivatization is, however, also done to give alkaloids better detection properties and for quantitative determinations.

TABLE 3.1

DERIVATIZATION REACTIONS AND REAGENTS USED BY GAS CHROMATOGRAPHY OF ALKALOIDS

Derivatization	Reagent	Alkaloid
TOBACCO ALKALOIDS		
Hydrogenation + pentafluoropropylation	Perfluoropropionic anhydride	nicotine ¹
Trichloroethylchlorocarbamate derivatization	Trichloroethyl chloroformate	nicotine ²
PYRROLIZIDINE ALKALOIDS		
Trimethylsilylation	N,N-bis(trimethylsilyl)trifluoroacetamide	pyrrolizidine alkaloids ³
TROPINE ALKALOIDS		
Trimethylsilylation	N,O-bis(trimethylsilyl)acetamide	scopolamine ^{4,8} , tropic acid ⁸ , scopoline ⁸
	Hexamethyldisilazane	tropine alkaloids ⁶
Heptafluorobutylation	N-methyl-N-trimethylsilyltri-fluoroacetamide	tropine, scopoline, atropine, scopolamine, meteloidine ⁵
	Heptafluorobutyric anhydride	scopoline ⁷
PSEUDOTROPINE ALKALOIDS		
Methylation	Diazomethane	benzoylecgonine ^{9,10}
	Dimethylformamide + dimethylacetal	benzoylecgonine ¹¹
Trimethylsilylation	N-methyl-N-trimethylsilyltri-fluoroacetamide	ecgonine, pseudoecgonine, benzoylecgonine ⁵
	N,O-bis(trimethylsilyl)acetamide	cis- and trans-cinnamoyl-cocaine, ecgonine, benzoylecgonine ¹⁰

TABLE 3.1 (continued)

Derivatization	Reagent	Alkaloid
Acylation	Hexafluoroisopropanol + heptafluorobutyric anhydride	ecgonine, benzoyl-ecgonine ¹⁵
Reduction and O-acylation	LiAlH ₄ + pentafluoropropionic or heptafluoropropionic anhydride	cocaine ¹⁴
CINCHONA ALKALOIDS		
Methylation	Trimethylanilinium hydroxide	quinidine ¹⁷
Trimethylsilylation	N-methyl-N-trimethylsilyltri-fluoroacetamide or bis(tri-methylsilyl) trifluoroacetamide	cinchona alkaloids ¹⁶
CACTUS ALKALOIDS		
Trifluoroacetylation	Trifluoroacetic anhydride	mescaline ¹⁸
EPHEDRA ALKALOIDS		
Acetone derivatization	Acetone	ephedrine, pseudo-ephedrine ^{19,20}
Trifluoroacetylpropylation	N-trifluoroacetyl-L-propyl chloride	ephedrines, pseudo-ephedrines ^{21,22}
N-(R)- α -phenylbutyryl-O-trimethylsilylation	N-(R)- α -phenylbutyric anhydride + N,O-bis(trimethylsilyl)acetamide	ephedrines, pseudo-ephedrines ²³
Oxidation	Sodium metaperiodate	ephedrine ^{24,25}
OPIUM ALKALOIDS		
Acetylation	Acetic anhydride	morphine ^{26,27,32,39} , morphine-N-oxide, nor-morphine, pseudomorphine, codeine ³⁹
Fluoroacetylation	Trifluoroacetic acid	morphine, codeine ³⁷
	Trifluoroacetic anhydride	morphine ^{39,41,49} , morphine-N-oxide, nor-morphine, pseudomorphine, codeine, nor-codeine ³⁹
Propionylation	Trifluoroacetylimidazole	morphine ⁵¹
	Propionic anhydride	morphine ^{26,27,56} , 6-O-acetylmorphine ⁵⁶
Pentafluoropropylation	Pentafluoropropionic anhydride	morphine ^{36,45,53,57} , codeine ^{36,57} , 6-O-acetyl-morphine ⁵⁷
Heptafluorobutylation	Heptafluorobutyric anhydride	6-O-acetylmorphine ^{52,54}
	Heptafluorobutyric acid	morphine, codeine ³⁷ , apo-morphine ⁴⁶
	Heptafluorobutyrylimidazole	morphine ⁵⁵

TABLE 3.1 (continued)

Derivatization	Reagent	Alkaloid
Trimethylsilylation	Hexamethyldisilazane	morphine ²⁸
	Hexamethyldisilazane + trimethylchlorosilane	morphine ²⁹
	N,O-bis(trimethylsilyl)acetamide	morphine ^{30,33,38,40,44} apomorphine ^{34,35}
	N,O-bis(trimethylsilyl)acetamide + trimethylchlorosilane	morphine ³¹
	N,O-bis(trimethylsilyl)-tri-fluoroacetamide + trimethylchlorosilane	morphine ^{39,42,43} , morphine-N-oxide, nor-morphine, pseudomorphine, codeine, nor-codeine ³⁹
		morphine ⁵⁰
Pentafluorobenzoylation	Pentafluorobenzoyl bromide	morphine ⁵⁰
APORPHINE ALKALOIDS		
Trimethylsilylation	N,O-bis(trimethylsilyl)acetamide + trimethylsilylimidazole + trimethylchlorosilane	aporphines, tetrahydroberberines, demethylated aporphines, demethylated tetrahydroberberines ⁵⁸
Trifluoroacetylation	Trifluoroacetic anhydride	aporphines ⁵⁹
AMARYLLIDACEAE ALKALOIDS		
Trimethylsilylation	N,O-bis(trimethylsilyl)acetamide	Amaryllidaceae alkaloids ⁶⁰ Erythrina alkaloids ⁶¹
INDOLE ALKALOIDS		
Trimethylsilylation	Hexamethyldisilazane	hydroxyl substituted N,N-dimethyltryptamines ⁶²
	N-methyl-N-trimethylsilyl-trifluoroacetamide	vincamine ^{65,66}
	N,O-bis(trimethylsilyl)fluoroacetamide	hydroxyl substituted Vinca-alkaloids ⁶⁷
	N,O-bis(trimethylsilyl)acetamide	physostigmine ⁶⁸ ajmaline ⁶⁸
Hydrolysis and methylation of the acids formed	Diazomethane	reserpine, rescinnamine ⁶³
ERGOT ALKALOIDS		
Trimethylsilylation	N,O-bis(trimethylsilyl)acetamide	LSD ^{70,71,72} , lysergic acid amides ⁷³ , agroclavine ⁷⁶
	N,O-bis(trimethylsilyl)tri-fluoroacetamide	LSD ⁷⁴

TABLE 3.1 (continued)

Derivatization	Reagent	Alkaloid
Trifluoroacetylation	Trimethylsilyldiethylamine + trimethylsilylimidazole	ergometrine ⁷⁵
	N,O-bis(trimethylsilyl)tri-fluoroacetamide + trimethylsilyldiethylamine + trimethylsilylchlorosilane	agroclavine ⁷⁶
	Trifluoroacetic anhydride	agroclavine ⁷⁶
STEROIDAL ALKALOIDS		
Methylation	Methyl sulphate + sodium hydride	solanine, demissine ⁷⁷
XANTHINE ALKALOIDS		
Methylation	Trimethylanilinium hydroxide	theophylline ^{78,81, 92} theobromine ⁷⁸
Ethylation	Ethyl iodide	theophylline ⁹⁵
Butylation	Tetra- <i>n</i> -butylammonium hydroxide	xanthines ⁷⁹ , theophylline ^{87,94}
Propylation	Dimethylformamide + di- <i>n</i> -butylacetal	theophylline ^{82,89}
	Butyl iodide	theophylline ⁸³
	Tetramethylammonium hydroxide + butyl iodide	theophylline ⁸⁸
	(N,N-dimethylacetamide)tetramethylammonium hydroxide + butyl iodide	theophylline ^{91,93}
	Dimethylformamide + dipropylacetal	theophylline ⁸⁶
	Tetrapropylammonium hydroxide	theophylline ⁸⁰
	Pentyl iodide	theophylline ⁸⁴
	Pentafluorobenzyl bromide	theophylline ^{85,95} , caffeine, theobromine ⁹⁵
	Pentafluorobenzyl chloride	theophylline ⁹⁰
IMIDAZOLE ALKALOIDS		
Acylation	Heptafluorobutyric anhydride	pilocarpine

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II. SPECIAL PART

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II.1. PYRROLIZIDINE, PYRIDINE, PIPERIDINE AND QUINOLIZIDINE ALKALOIDS

Chapter 4

PYRROLIZIDINE ALKALOIDS

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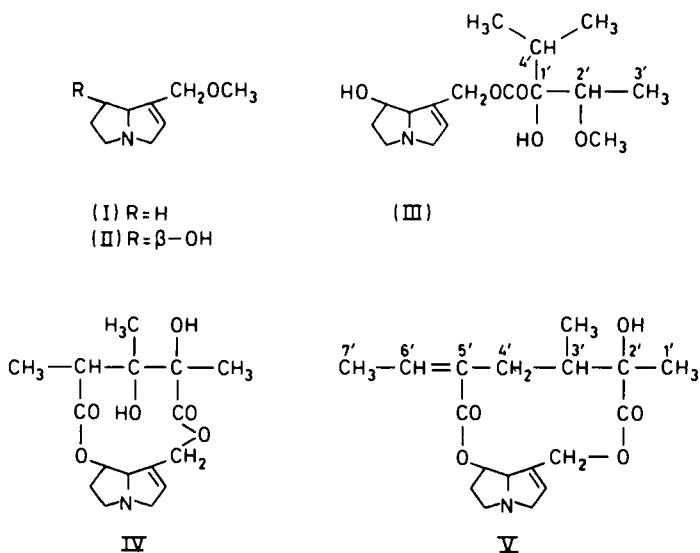
4.1 PYRROLIZIDINE ALKALOIDS

In a systematic study on gas chromatography of pyrrolizidine alkaloids, Chalmers et al.¹ emphasized the importance of using a silanized all-glass system and well deactivated support material to reduce the possibilities of adsorption and decomposition during gas chromatography. On a 6 ft. long silanized glass column packed with 4 % SE-30 on GasChrom P, the non-ester alkaloids could be gas chromatographed at 140°C, and the esters with monocarboxylic acids and the macrocyclic diester alkaloids at 205°C. The structures of some pyrrolizidine alkaloids are given in Figure 4.1.

FIGURE 4.1

PYRROLIZIDINE ALKALOIDS

I = 1-Methoxymethyl-1,2-dehydropyrrolizidine, II = its 7β-hydroxy derivative, III = heliotrine, IV = monocrotaline, V = senecionine



The retention times of the non-ester alkaloids and derivatives are listed in Table 4.1 and those of the esters with monocarboxylic acids in Table 4.2. In Table 4.3 the retention times of the macrocyclic diesters are given.

TABLE 4.1

RETENTION TIMES, t_R , OF NON-ESTER PYRROLIZIDINE ALKALOIDS AND DERIVATIVES ON A 4 % SE-30 PACKED COLUMN ON GAS CHROM P AT 140°C¹

Alkaloid	B.p. (°C/mm)	M.p. (°C)	t_R (min)
1-Methylenepyrrolizidine	115/150	-	1.6
Heliotridane (1 β -methyl-8 α -pyrrolizidine)	169/760	-	1.8
Anhydroplatynecine	194/750	-	2.4
7 β -Hydroxy-1-methylene-8 α -pyrrolizidine	41/0.1	35-36	3.3
Desoxyretronecine (7 β -hydroxy-1-methyl-1,2-dehydro-8 α -pyrrolizidine)	-	79-80	3.4
7 β -Hydroxy-1-methylene-8 β -pyrrolizidine	62/0.03	34-36	4.4
Retronecanol (7 β -hydroxy-1 β -methyl-8 α -pyrrolizidine)	140/30	98-98.5	4.5
Hydroxyheliotridane (7 α -hydroxy-1 β -methyl-8 α -pyrrolizidine)	92/0.5	61.5-62.5	4.5
7 α -Hydroxy-1-methyl-1,2-dehydro-8 α -pyrrolizidine	114/3.5	67-68	4.5
1-Methoxymethyl-1,2-dehydro-8 α -pyrrolizidine	100/10	-	5.4
1-Methoxymethyl-1,2-epoxypyrrolizidine	53/0.1	-	6.4
Isoretronal (1 β -hydroxymethyl-8 α -pyrrolizidine)	115-16/1.5	39-40	7.4
Supinidine (1-hydroxymethyl-1,2-dehydro-8 α -pyrrolizidine)	90/0.1	29-30	7.4
1-Hydroxymethyl-1,2-epoxy-8 α -pyrrolizidine	80/0.04	-	8.3
7 β -Hydroxy-1-methoxymethyl-1,2-dehydro-8 α -pyrrolizidine	77/0.4	36-38	9.0
7 β -Acetoxy-1-methoxymethyl-1,2-dehydro-8 α -pyrrolizidine	-	-	15.0
Retronecine (7 β -hydroxy-1-hydroxymethyl-1,2-dehydro-8 α -pyrrolizidine)	-	117-118	15.0
Heliotridine (7 α -hydroxy-1-hydroxymethyl-1,2-dehydro-8 α -pyrrolizidine)	-	117-118	15.0
Platynecine (7 β -hydroxy-1 β -hydroxymethyl-8 α -pyrrolizidine)	-	148-148.5	15.0

TABLE 4.2

RETENTION TIMES OF PYRROLIZIDINE ESTERS WITH MONO CARBOXYLIC ACIDS ON A 4 % SE-30 PACKED COLUMN ON GAS CHROM P AT 205°C¹

Alkaloid	M.p. (°C)	t_R (min)
7-Angelylretronecine	76-77	4.2
7-Angelylheliotridine	116-117	4.8
Heleurine	67-68	8.0
Supinine	148-149	8.8
Heliotrine	128	12.4
Indicine	97-98	14.3
Retronecine trachelanthate	-	14.3
Retronecine viridiflorate	-	14.3
Rinderine	100-101	15.6
Echinatine	109-110	15.6
Europine	(N-oxide 171)	18.2
Sarracine	45-46	29.1

TABLE 4.2 (continued)

Alkaloid	M.p.(°C)	t _R (min)
Echiumine	99-100	35.6
Lasiocarpine	96.5-97	46.4
Echimidine	(picrate 142-143)	47.6
Heliosupine	(picrate 103-106)	50.6
Latifoline	102-103	55.0

TABLE 4.3

RETENTION TIMES OF MACROCYCLIC DIESTER ALKALOIDS ON A 4 % SE-30 PACKED COLUMN ON GAS CHROM P AT 205°C¹

Alkaloid	M.p.(°C)	t _R (min)
Retusine	174-175	13.9
Fulvine	213.5-214	15.5
Crispatine	137-138	15.6
Monocrotaline	202-203	19.5
Senecionine	245	20.6
Seneciophylline	217	21.1
Platyphylline	129	24.0
Integerrimine	172.5	24.3
Spectabiline	185.5-186	27.2
Senkirkine	198	31.6
Jacobine	228	34.0
Sceleratine	178	34.6
Jacozine	228	35.5
Jacoline	221	36.6
Rosmarinine	209	37.1
Jaconine	147	40.4
Retrorsine	219-220	40.7
Riddelline	198	41.9
Retusamine	174.5	50.3
Otosenine	221	51.0
Grantianine	209-209.5	57.0

Stengl et al.² carried out an investigation of the liver toxic pyrrolizidine alkaloids in *Symphytum officinale* by means of gas chromatography. The alkaloid mixture was extracted in a Soxhlet apparatus with methanol, the extract purified by repeated acidic aqueous and alkaline organic solvent extraction and column chromatography on Florisil. After derivatization with *n*-butylboronic acid and N,N-bis(trimethylsilyl)trifluoroacetamide the alkaloids were gas chromatographed on a 1.8 m by 2 mm I.D. glass column packed with 4 % SE-30 on Varaport (80-100 mesh). Five alkaloids, lycopsamine, intermedine, acetyllycopsamine, acetyl-intermedine and symphytine were identified and their amounts quantitatively determined. The gas chromatographic separation of the alkaloid derivatives is shown in Table 4.4.

TABLE 4.4

RETENTION TIMES OF *SYMPHYTUM OFFICINALE* ALKALOIDS²

after derivatization with *n*-butylboronic acid and *N,N*-bis(trimethylsilyl)trifluoroacetamide on a packed column with 4 % SE-30 and temperature programming from 200°C to 300°C

	t_R (sec)	t_R (rel)
Symphytine	779	0.698
Acetyllycopsamine	576	0.516
Acetylintermedine	598	0.536
Lycopsamine	545	0.489
Intermedine	562	0.504
Int. standard (Cholesterol TMS)	1116	1.000

TABLE 4.5

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF PYRROLIZIDINE ALKALOIDS

Column	Solid support	Stat.phase %	Temperature	Comp.Preparation	Ref.
glass, 6 ft x 6 mm I.D.	Gas Chrom P	SE-30	4	140°C non ester alk.	1
glass, 1.8 m x 2 mm I.D.	Varaport	SE-30	4	205°C ester alk. 200-300°C pr.	2

Abbreviations: alk = alkaloid(s), pr = (temperature) programming

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Chapter 5

PYRIDINE ALKALOIDS

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5.1 TOBACCO ALKALOIDS

The first group of alkaloids that was subjected to gas chromatographic analysis was the very volatile tobacco alkaloids. They were gas chromatographed by Quin¹ in 1958 on packed columns with polyglycols as stationary phases. Since then a great number of papers have been published of tobacco alkaloids in connection with

- 1) studies on the alkaloidal composition of various types of tobacco,
- 2) studies on the alkaloids in tobacco smoke and particulate matter of tobacco smoke,
- 3) studies on the biosynthesis of tobacco alkaloids,
- 4) studies on the metabolism of tobacco alkaloids in man,
- 5) determination of nicotine in blood, tissue and urine, as well as in
- 6) residues in food.

Investigations by means of gas chromatography have also been conducted to

- 7) study the thermal decomposition of tobacco alkaloids.

To increase the sensitivity of the method, derivatization of nicotine has been done with the use of an electron capture detector. Also, multiple ion detection, employing deuterated alkaloids as internal standards, has been used for the same purpose.

A number of different stationary phases have been used, mostly on a solid support impregnated with potassium hydroxide; from very polar ones, such as polyglycols, to non-polar ones, such as silicone rubber. Packed columns have mostly been used, but good separations have also been achieved with capillary columns.

Many investigations have been carried out of gas chromatography of tobacco alkaloids in order to study the volatile compounds of the smoke, *i.e.* thermal decomposition products of alkaloids and other substances in tobacco. This chapter is mainly concerned with the gas chromatography of the alkaloids themselves and their metabolites in man, also to some extent with the decomposition products of the alkaloids.

In his first publication on gas chromatography of tobacco alkaloids Quin¹ used packed columns with polyethylene glycol, polypropylene glycol and polybutylene glycol as stationary phases on alkali-washed firebrick. The percentage of stationary phase was 25. The polyglycol columns exhibited good selectivity for the alkaloids, making the separation of most of the members of complex mixtures possible, as shown in Table 5.1.

TABLE 5.1

SEPARATION OF TOBACCO ALKALOIDS ON POLYGLYCOL COLUMNS, 25 % ON ALKALI-WASHED FIREBRICK¹

Liquid phase	Columns and conditions		
	Polyethylene glycol	Polypropylene glycol	Polybutylene glycol
Temperature	190°C	190°C	180°C
Helium flow (ml/min)	48	45	50
	Retention times (min)		
3-Pyridyl methyl ketone	4.3	4.3	3.1
3-Pyridyl ethyl ketone	5.3	6.1	5.0
3-Pyridyl <i>n</i> -propyl ketone	6.6	8.1	7.0
Nicotine	5.2	8.6	8.2
Nor-nicotine	12.3	16.1	14.3
Myosmine	13.4	16.4	14.7
Anabasine	13.8	19.4	18.1
Metanicotine	16.5	23.5	20.9
Nicotyrine	19.4	21.0	18.3
Cotinine	85	79	63

To control whether or not the structure of the alkaloids had been altered during the gas chromatography, Quin isolated the compounds eluted from the column and showed that in all cases the collected and starting compounds were identical.

The method was applied for the analysis of the alkaloids in tobacco smoke^{2,3}. To overcome difficulties associated with the wide boiling range of the alkaloidal mixture and the relatively massive amount of nicotine present, the gas chromatography was carried out under three sets of conditions: 1) At 140-150°C on a 1 m by 6 mm column for the alkaloids emerging before nicotine; 2) at 190°C on a 1 m by 10 mm column for the alkaloids immediately following nicotine. The wide diameter permitted a large nicotine load to be placed on the column without an asymmetric, tailing peak resulting; 3) at 190°C on a 1 m by 6 mm column for the higher boiling alkaloids.

For routine determinations of nicotine and nornicotine in tobacco samples Quin and Pappas⁴ extracted the alkaloids with benzene-chloroform (1:1) from tobacco made alkaline with sodium hydroxide. Nicotine was determined on a 1 m long polypropylene glycol column 25 % at 190°C and nornicotine on a 1 m long polybutylene glycol column 25 % at 180°C. The nornicotine determination requires a column specially selected to give good resolution from anabasine. Myosmine, which may be present in some tobacco samples to an extent that may interfere with the nornicotine determination, can be detected by using a 2 m long polyethylene glycol column operated at 150°C. The method described is most useful when the amount of alkaloids exceeds about 0.2% of the tobacco. The reproducibility of the method is good, as can be seen in Table 2.

In connection with studies on the thermal decomposition of tobacco alkaloids Kobashi⁵ investigated the separation and identification of bases of nicotine and pyridine. For the nicotine bases and related high boiling pyridine bases (see Table 5.3) the separation was carried out using silicone grease and polyethylene glycol 6000 columns treated with potassium hydroxide. The retention times of these compounds related to nicotine on the columns mentioned are given in Table 5.3, together with the boiling points of each compound.

Kobashi and Watanabe⁶ carried out determinations of pyridine and nicotine homologs using packed columns with polyethylene glycol 1500 and 6000 and support pretreated with potassium

hydroxide. The determinations of the alkaloids' calibration curves, showing the relation between the ratio of peaks' areas and that of the alkaloids' weight were made to within 1.5 % error.

TABLE 5.2

ALKALOIDAL CONTENT OF SEVERAL TOBACCOS⁴

Sample	Nicotine %			
	Burley	Bright	Commercial Cigarette	Cigar Filter
1	2.04	1.68	1.28	0.59
2	2.06	1.72	1.34	0.62
3	2.06	1.68	1.24	0.56
4	1.93	1.67	1.25	0.59
5	1.93	1.67	1.31	0.62
Average	2.00	1.68	1.29	0.60
Std. dev.	0.07	0.02	0.04	0.03

	Nornicotine %		
	<i>Nicotiana silvestris</i>	Cherry Red	<i>Nicotiana tabacum</i>
1		1.53	1.41
2		1.56	1.41
3			1.41
Average		1.55	1.41

TABLE 5.3

RELATIVE RETENTION TIMES OF NICOTINE BASES AND HIGH BOILING PYRIDINE BASES⁵

Compound	B.p. (°C)	Relative retention times	
		PEG 6000	Silicone grease
α -Aminopyridine	211	0.89	0.27
β -Pyridyl methyl ketone	218	0.88	0.39
β -Pyridyl <i>n</i> -propyl ketone	245	1.24	0.77
Nicotine	247	1.00 (10.6 min)	1.00 (11.3 min)
N-methyl anabasine	121/7 mm Hg	1.55	1.44
β -Aminopyridine	250	2.00	0.39
Dihydrometanicotine	141/15 mm Hg	2.00	1.46
Nornicotine	267	2.30	1.24
Myosmine	118/3.2 mm Hg	2.47	1.26
Anabasine	146/15 mm Hg	2.59	1.72
Anatabine	145/10 mm Hg	4.60	1.60
α, α' -Dipyridyl	272	2.78	1.37
Metanicotine	110/1.5 mm Hg	2.87	1.64
Nicotyrine	280	3.47	1.45
α, β' -Dipyridyl	137/4 mm Hg	5.50	1.83
N-methyl nicotineamide		10.30	1.73
Cotinine	210/6 mm Hg	14.20	3.10

In a paper on the mechanism of demethylation of nicotine, Craig et al.⁷ used gas chromatography to separate and estimate the alkaloidal products from the reaction mixture. They obtained good separation on a 2 m long column using PEG 20M 5.6 % as stationary phase on Firebrick, at 200°C. The retention times of the individual alkaloids are given in Table 5.4.

TABLE 5.4

RETENTION TIMES OF INDIVIDUAL TOBACCO ALKALOIDS ON A 2 M LONG PEG 20M 5.6 % COLUMN AT 200°C; QUIN: PEG 20M, 190°C, NICOTINE 5.2 MIN; KOBASHI: PEG 6000, 220°C, NICOTINE 10.6 MIN⁷

	Time (min)	Ratio	Quin ratio	Kobashi ratio
3-Pyridyl ethyl ketone	17.28	1.00	1.01	
3-Pyridyl <i>n</i> -propyl ketone	21.92	1.26	1.27	1.24
Nicotine	17.28	1.00	1.00	1.00
N-Methylmyosmine	25.76	1.49		
Nornicotine	39.68	2.30	2.37	2.30
Myosmine	42.88	2.49	2.57	2.47
Anabasine	43.52	2.52	2.65	2.57
Metan nicotine	45.92	2.66	3.17	2.87
Nicotyrine	58.88	3.42	3.73	3.47
N-Methylnicotineamide	179.36	10.47	12.3	10.3
Cotinine	239.20	13.84	16.4	14.2

Weeks et al.⁸ compared the separation of nicotine, nornicotine, myosmine, anabasine and anatabine on three gas chromatographic packed columns using SE-30, Versamid and DC 550 as stationary phases. Relative retention times, effective plate values and resolution are listed in Table 5.5.

TABLE 5.5

RELATIVE RETENTION TIME (t_R), EFFECTIVE PLATE VALUES (N) AND RESOLUTION (R) OF TOBACCO ALKALOIDS⁸.

2.44 m long columns packed with 10 % DC 550, 5 % SE-30 or 10 % Versamid 900 on Chromosorb W, column temperatures 170°C, 190°C and 170°C and carrier gas flow 40, 30 and 40 ml/min respectively.

Alkaloid	DC 550			SE-30			Versamid 900		
	t_R	N	R	t_R	N	R	t_R	N	R
Nicotine	1.00	2532	5.12	1.00	1390		Nicotine	1.00	1258
Nornicotine	1.55	2320	1.24	1.21	1366	1.57	Myosmine	1.97	1290
Myosmine	1.87	-	0.64	-	-	1.20	Nornicotine	2.15	1330
Anabasine	2.03	2511	2.18	1.41	1302		Anabasine	2.60	1642
Anatabine	2.50	2000		-	-		Anatabine	3.28	1580

Massingill and Hodgkins⁹ in a study on the gas chromatographic separation of alkaloids, used four packed columns with JXR (dimethylpolysiloxane), SE-52, XE-60 and Epon 1001 resin as stationary phases, and capillary columns with QF-1, SE-30 and Apiezon L. Nicotine and anabasine were well separated on SE-52, JXR and XE-60 packed columns. On the capillary columns nicotine and anabasine were well separated on the Apiezon L column at 125°C, but tailing was pronounced. The SE-30 capillary column resolved the two alkaloids mentioned, with some tailing; the 100 foot QF-1 column gave good resolution with sharp peaks and practically no tailing. Although the 100-foot QF-1 capillary column out-performed the other capillary columns and gave good resolution of the alkaloids with molecular weight of up to 303, a comparison of the separation of *i.a.* atropine-homatropine-scopolamine showed that the packed columns gave better resolution (a 6 foot x 1/8 inch 1 % QF-1 packed column and a 100 foot QF-1 capillary column).

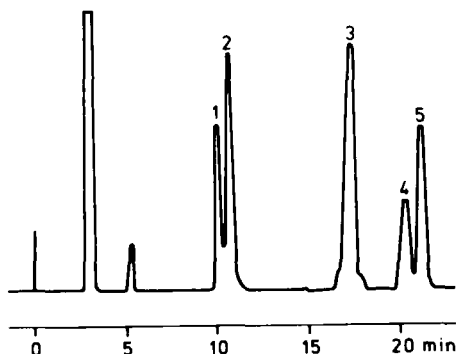
Harke and Drews¹⁰ separated nicotine, nornicotine, anabasine and nicotyrine on a 50 m long

by 0.5 mm steel capillary coated with Ucon LB 550X (= polypropylene glycol) and KOH. However, myosmine and nornicotine could not be separated under the conditions given. A typical chromatogram is shown in Figure 5.1.

FIGURE 5.1

GAS CHROMATOGRAM OF TOBACCO ALKALOIDS¹⁰

1 = Pyridyl-*n*-propylketon, 2 = Nicotine, 3 = Nornicotine and Myosmine, 4 = Anabasine, 5 = Nicotyrine



To be able to determine nicotine in sub-picomole quantities Neelakantan and Kostenbauer¹¹ used an electron-capture detector in connection with derivatization of nicotine: Nicotine was hydrogenated catalytically to yield *N*-methyl-4-(3'-piperidyl)-*n*-butylamine (= octahydronicotine), the two secondary amino-functions of which were treated with perfluoropropionic anhydride to give the di-pentafluoropropionyl octahydronicotine. The perfluoropropionyl derivative of octahydronicotine provides an electron capture derivative which represents a means of detecting nicotine in amounts of approximately 1/100th of the minimum quantity of nicotine that is detectable by FID.

For the analysis of nicotine in the picogram range, Hartvig et al.¹² prepared the δ -tri-chloroethylcarbamate derivative of nicotine, which has an excellent sensitivity in the electron-capture detector. The adsorptive properties of the derivative are lower than those of nicotine during gas chromatography. However, the formation of the corresponding olefin due to partial thermal dehydrohalogenation in the injector and on the column is a disadvantage of the procedure. Nicotine is subjected to reaction at 90°C with trichloroethyl chloroformate in the presence of pyridine to form the carbamate, in which the pyrrolidine ring is opened. On heat treatment (*i.e.* in the injector) the carbamate partially formed the corresponding olefin. For quantitative determination *N*-*n*-propylornicotine was used as an internal standard. The precision at the 30 ng/ml level was $\pm 8.8\%$ ($n = 7$).

5.1.1. Alkaloids in tobacco samples

In 1962 Quin and Pappas⁴ used gas chromatography for the routine determination of nicotine and nornicotine in tobacco samples. Nicotine was determined on a 1 m long propylene glycol column 25 % at 190°C, and nornicotine on a 1 m long polybutylene glycol column 25 % at 180°C. The nornicotine determination required a specially selected column to give resolution from anabasine. Myosmine, which may be present in some tobacco samples to an extent that may interfere with the nornicotine determination, was detected by using a 2 m long polyethylene glycol column operated at 150°C.

Yasumatsu and Murayama¹³ used polyethylene glycol 20 M as stationary phase for the determination of nicotine in tobacco samples. The alkaloids were extracted with 0.5 N HCl containing isoquinoline as an internal standard. The mixture of the extract and an equivalent volume of N NaOH was injected into the injector of the gas chromatograph fitted with a soda lime tube. The nicotine content was determined from the peak heights of nicotine and isoquinoline. The relative standard deviation was 1.9 %.

In a paper published in 1971, Yasumatsu and Murayama¹⁴ improved the technique and the gas chromatographic conditions for the determination of nicotine.

Bush¹⁵ determined the four most important tobacco alkaloids (nicotine, nornicotine, anabasine and anatabine) using a 10 % DC 550 packed column on Chromosorb 60-80, and using isoquinoline as an internal standard. The alkaloids were extracted with benzene-chloroform (9:1) after treatment of the tobacco sample (1 g) with bariumhydroxide and water. The organic phase was concentrated and used for the gas chromatographic determination. Because of the great differences in the amounts of the minor alkaloids and nicotine in most tobacco samples, two extractions, each with an appropriate amount of internal standard, were required for a complete assay. The precision of the quantitative analysis on tobacco samples of different alkaloids is given in Table 5.6.

TABLE 5.6

PRECISION OF QUANTITATIVE ALKALOID ANALYSIS ON TOBACCO SAMPLES OF DIFFERENT ALKALOID COMPOSITION¹⁵

Sample	Nicotine (mg/g)	Nornicotine (µg/g)	Anabasine (µg/g)	Anatabine (µg/g)
1	20.14 ± 0.19	393 ± 12.0	65 ± 3.0	408 ± 7.0
2	9.79 ± 0.26	(47.6 ± 1.53) · 10 ³	trace	(2.39 ± 0.13) · 10 ³
3	(264 ± 4.48) · 10 ³	1268 ± 45.6	not detected	35.3 ± 1.40

A quantitative comparison of alkaloid analysis using gas chromatography and steam distillation is given in Table 5.7.

A rapid, accurate and reproducible method to determine very low nicotine levels in tobacco (0.03 % - 3 %) was developed by Lyerly and Greene¹⁶. Samples of 1 g tobacco were treated with sodium hydroxide solution and the alkaloids extracted with chloroform. Nicotine in the chloroform was determined by gas chromatography on a Castorwax-KOH packed column, using *n*-hexadecane as an internal standard. The results obtained agreed quite well with the results from other methods, especially the picrate method.

To determine certain alkaloids, other than nicotine, in tobacco samples (nornicotine,

TABLE 5.7

QUANTITATIVE COMPARISON OF ALKALOID ANALYSIS USING GAS CHROMATOGRAPHY AND STEAM DISTILLATION¹⁵

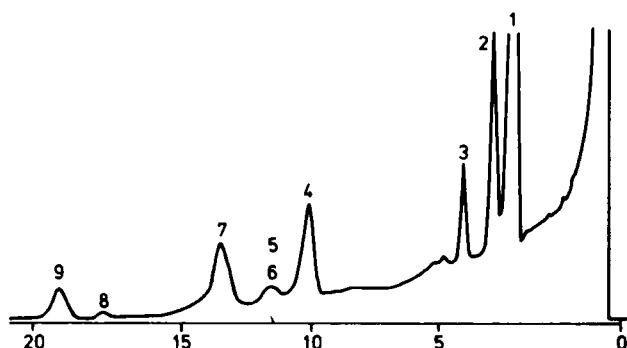
Sample	Gas chromatography					Steam distil- distillation Total (mg)
	Nicotine (mg/g)	Nornicotine (μ g/g)	Anabasine (μ g/g)	Anatabine (μ g/g)	Summation (mg)	
Tobacco	20.4 \pm 0.2	642 \pm 32	109 \pm 5	541 \pm 8	21.69	20.25
Tobacco + low-level exogenous alkaloids	30.6 \pm 0.9	709 \pm 20	304 \pm 7	768 \pm 16	32.38	31.40
Tobacco + high-level exogenous alkaloids	40.0 \pm 0.8	1120 \pm 35	549 \pm 11	954 \pm 18	42.62	40.80

nicotyrine, anatabine, anabasine/myosmine) Burns and Collin¹⁷ developed a method using a packed column with Carbowax 20 M and KOH as stationary phase. No separation was obtained for anabasine and myosmine. Samples of 2 g tobacco were extracted by Soxhlet extraction with methanol. Quinaldine (= 2-Methylquinoline) was added as an internal standard. The amounts of alkaloids were expressed as nornicotine. A typical chromatogram of a tobacco extract is shown in Figure 5.2.

FIGURE 5.2

GAS CHROMATOGRAM OF A TYPICAL TOBACCO EXTRACT¹⁷

1 = Nicotine, 2 = unknown, 3 = internal standard, 4 = nornicotine, 5 + 6 = anabasine + myosmine, 7 = solvent impurity, 8 = nicotyrine, 9 = anatabine

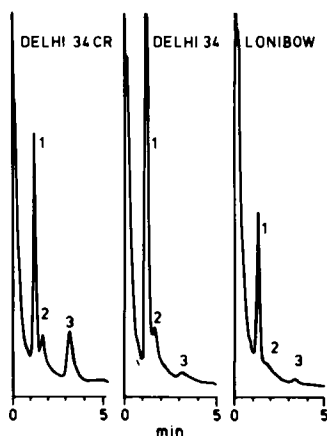


When the major alkaloid in tobacco samples is nor-nicotine, the commonly used steam-distillation method and automated procedures result in poor estimates of nicotine and nornicotine. Rosa¹⁸ therefore developed a pyrolysis-gas chromatographic method, whereby pyrolysis was carried out with a Victoreen pyrolyzer fitted to the gas chromatograph. Nicotine is relatively volatile and readily released by pyrolysis, even at 100°C. Nornicotine, being less volatile, showed maximum release by pyrolysis at 300°C. The pyrolysis-gas chromatography was carried out with ca. 1 mg of tobacco. The results obtained with the method are presented in Figure 5.3.

FIGURE 5.3

QUALITATIVE COMPARISON OF PYROLYSIS-GAS CHROMATOGRAPHY OF THREE TOBACCO CULTIVARS¹⁸

Ca. 1 mg of tobacco tissue was used to produce each pyrogram; 1 = Nicotine, 2 = Neophytadiene, 3 = Nornicotine.



The pyrolysis-gas chromatography offers a reliable and rapid means of estimating nornicotine in tobacco without extraction.

Although Massingill and Hodgkins² had demonstrated in 1965 that tobacco alkaloids could be analyzed on capillary columns, several years passed before capillary gas chromatography of tobacco alkaloids became more commonly used. Severson et al.¹⁹ developed a method for the determination of alkaloids in tobacco samples and fresh tobacco leaves, involving a brief sonification-extraction step with subsequent capillary gas chromatographic separation and quantification. The method permitted the screening of numerous samples using a Nitrogen-Phosphorus-detector, giving a linear and very reproducible response. However, when such a detector was replaced by another, each was found to give a relative response against concentration, so that a new calibration curve had to be set up. As little as 25 mg of ground tobacco was extracted, with 1 ml 0.05 N methanolic potassium hydroxide containing the internal standard (0.25 mg/ml of 2,3' Dipyridyl), by sonification, and with 1 μ l samples injected. A 35 m x 0.25 mm I.D. glass capillary coated with Carbowax (temp. programming 170-200°C - 2°C/min) and a 15 m x 0.25 mm I.D. glass capillary coated with SuperoxTM (temp. programming 130-200°C - 4°C/min) were used.

5.1.2. Alkaloids in tobacco smoke

Quin^{2,3} was the first to apply gas chromatography for the analysis of nicotine alkaloids in tobacco smoke. He used three sets of conditions to analyse the many alkaloids present in a wide boiling range.

Lyerly and Greene¹⁶ determined nicotine, menthol and some other non-alkaloidal constituents in tobacco smoke. Single cigarettes were smoked through a Cambrigde filter, and the filter

placed in a specially designed holder, which was heated while allowing carrier gas to flow through it. The vapors were collected and deposited on the chromatographic column. The procedure permits a puff-by-puff analysis. Jacin et al.²⁰ developed a method for the determination of nicotine in tobacco samples, and in the particulate matter of smoke. In the latter case the Cambridge filter, through which the smoke of five cigarettes had been smoked, was extracted with benzene-chloroform (9:1), and the extract was gas chromatographed on a packed column with neopentyl glycol adipate as stationary phase. Comparison with a spectrophotometric method showed that the gas chromatographic method was at least as good as the spectrophotometric method. It was also very simple and rapid.

Anastasov et al.²¹, using a packed column with 20 % Apiezon L on Chromosorb, found nicotine, nornicotine, myosmine, anabasine and metan nicotine, as well as a number of pyridine bases, in tobacco smoke.

A rapid method for the determination of nicotine in the particulate matter of tobacco smoke was developed by Yasumatsu et al.²². In a propyl alcohol extract of particulate matter of tobacco smoke retained on a glass fiber filter, the nicotine content was determined on a 25 % PEG 20 M column on Celite 545 at 190°C. Analysis of a plain and a filter cigarette gave 2.34-2.52 and 1.07-2.15 mg nicotine, respectively. Also, Kusama et al.²³ used gas chromatography to determine nicotine (and tar) in cigarette smoke, using a column of 5 % Castor wax on Diasolid L 60-80 mesh, 1.5 m long by 3 mm, at 160°C. The internal standard was *n*-hexadecane. There were no significant differences between the analytical results of nicotine in cigarette smoke obtained by gas chromatography and that by a UV-method. However, the coefficients of variation of the results obtained by gas chromatography were considerably lower than those obtained by UV-spectrophotometry.

Ohnishi et al.²⁴ determined the nicotine in cigarette filters and in the main-stream smoke adsorbed by a filter by extraction with isopropyl alcohol containing *n*-hexadecane as an internal standard. A packed glass column, 2 m long by 3 mm with Castor wax 5 % as stationary phase on Diasolid L 60-80 mesh at column temperature 150°C, was used. Also, Randolph²⁵ used extraction of Cambridge filters with dry isopropyl alcohol to determine the nicotine content of the particulate matter of smoke. He used a packed column with Carbowax 20 M-polyphenylether on Gas Chrom Q treated with 2 % KOH. The retention times of various tobacco alkaloids are given in Table 5.8. No internal standard was used by the quantitative determinations. Recoveries of nicotine over a wide range of concentrations averaged 98 %, when nicotine was added to the standard blended cigarette.

TABLE 5.8

RETENTION TIMES OF VARIOUS TOBACCO ALKALOIDS

6 ft long packed column (Carbowax 20 M 7 %, Polyphenyl ether (6 ring) 3 %, KOH 2 % on Gas Chrom Q 80-100 mesh) at 190°C²⁵

Nicotine	2 min 50 sec
Anabasine	6 min 51.6 sec
Nor-nicotine	6 min 0.8 sec

Hollweg et al.²⁶ determined simultaneously nicotine and water in tobacco smoke condensates. For the nicotine determination a packed column, 1.8 m long by 2 mm I.D. with 10 % Carbowax 20 M on Chromosorb WHP 60-80 mesh was used. Since the column was prepared without addition of

alkali, it lasted longer. No tailing of the nicotine peak was observed, when small amounts of the sample were injected. Good reproducibility was achieved.

5.1.3. Nicotine in urine

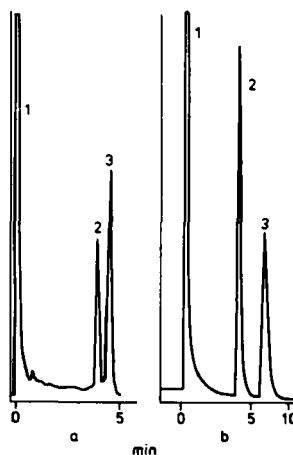
Several investigations have been carried out to study the content of nicotine in urine, in blood and in biological tissue in general. McNiven et al.²⁷ extracted the urine at pH 1 with methylene chloride to remove neutral and acidic material, and at pH 11 with the same solvent to isolate the nicotine. Back extraction with aqueous hydrochloric acid followed by basification and extraction with acetonitrile gave a solution containing the nicotine. It was gas chromatographed on an SE-30 column at 200°C using 3-methyl-3-phenylpiperidine as an internal standard. Recovery ranged from 60 to 95 %.

Beckett and Triggs²⁸ were interested in the determination of nicotine and its main metabolite cotinine in urine. An acidified urine was extracted with diethyl ether to remove impurities, the urine was made alkaline with sodium hydroxide and the nicotine extracted with diethyl ether. Cotinine was extracted from urine with methylene chloride after basification of the sample with ammonia. On concentration of the solution, the gas chromatography was carried out on a packed column treated with KOH using Carbowax 20 M as stationary phase. A relative recovery of 95-100 % for the two alkaloids was obtained with respect to their internal standard, chlorophenthermine for nicotine and lignocaine for cotinine, which were added to the urine at the start of the assay procedure. Typical chromatograms are given in Figure 4.

FIGURE 5.4

CHROMATOGRAMS OF TOBACCO ALKALOIDS FROM URINE²⁸

- a) 1 = diethyl ether, 2 = nicotine,
3 = chlorophenthermine
b) 1 = methylene chloride, 2 =
lignocaine, 3 = cotinine



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The same experimental conditions were used by Beckett et al.²⁹ for further studies of nicotine metabolism in man. However, phendimetrazine was used as the internal standard for nicotine, and lignocaine for cotinine.

For the determination of nicotine in urine, Cano et al.³⁰ used packed columns of different

kinds using firebrick, Chromosorb and Gas Chrom Q as solid support - all treated with 2-6 % KOH and Apiezon, Versamid 900 and Ucon 50 HB 2000 Polar as stationary phases. Firebrick columns gave symmetrical peaks, but the number of theoretical plates was low; Chromosorb W gave high numbers of plates, but tailing peaks. Best results were obtained with the Gas Chrom Q - KOH (6 %) - Ucon 50 HB 2000 Polar column. For the application of the gas chromatographic method for determination of nicotine in urine, the sample (30 ml) was made acid and extracted with diethyl ether, then basified with NaOH (pH 12.5) and extracted with diethyl ether. After concentration, the ether solution was used for gas chromatographic determination. Recovery: $90 \pm 3 \%$.

The same method was used by Cano et al.³¹ for the determination of nicotine in air and in the urine of smokers. Air was pumped through 30 ml sulphuric acid 2N and 30 ml urine was used for one analysis, which was carried out as described above.

The method described by Cano et al.^{30,31} was modified by Dumas et al.³², the length of the column was reduced from 1.5 m to 1 m. Two column temperatures were used, 120°C and 180°C, for the determination of nicotine and cotinine, respectively. For the extraction of the urine samples (20 ml) chloroform was used instead of diethyl ether. Recovery was, for nicotine, $90 \pm 4 \%$. Dumas et al.³³ also developed a micro-method for the same determination in urine and blood. Samples of 3 ml blood were treated with ammonium oxalate and filtered. 1 ml plasma was made alkaline and extracted with chloroform three times, the chloroform extract was evaporated and the residue solved in 10 μ l of a solution of 5 ng diphenylamine/ μ l (internal standard) in ethyl acetate. The solution was used for the gas chromatographic assay. A specific N-detector was used and the sensitivity was 0.2 ng for nicotine and 1 ng for cotinine. The recovery is given in Table 5.9.

TABLE 5.9

RECOVERY OF NICOTINE AND COTININE³³

Sample	Nicotine			Cotinine		
	added ng	found ng	%	added ng	found ng	%
I	20	17.5	87.5	20	18.8	94
II	40	38.08	95.2	40	38.8	97.2
III	40	36.84	91.1	40	38.0	95.2

Tausch et al.³⁴ found that gas chromatography of trace amounts of nicotine and its metabolites required capillary columns of very high inertness against basic compounds. Borosilicate glass columns contain relatively high amounts of B_2O_3 and Al_2O_3 , which act as strong Lewis acids. By leaching the columns with aqueous HCl, such compounds can be removed. Subsequent high temperature silylation and coating with a non polar silicone gum phase like OV-1 or SE-30 yielded columns of low adsorptivity for i.a. tobacco alkaloids. The columns were highly thermostable and long-lived unless water or large amounts of impurities were introduced into the capillary. To obtain sufficiently inert columns for routine analysis of such relatively impure extracts, the acidic leaching was followed by forming of a $BaCO_3$ intermediate layer. This pretreatment permitted coating with polyethylene glycol phases like Carbowax 20 M or Pluronic F 68. These columns exhibited besides satisfactory inertness a considerable higher durability in routine analysis of plasma and urine extracts.

5.1.4. Nicotine in blood and tissue

Schievelbein and Grundke³⁵ developed an assay for the determination of nicotine in blood and tissue. The proteins were removed from blood samples by treating with trichloroacetic acid. The filtrate was made alkaline with NaOH. MgO and NaCl were added and it was distilled to dryness. The distillate was collected, made acid and the solvent evaporated. NaOH-solution and acetonitrile were added and the acetonitrile solution used for gas chromatography. Tissue samples were homogenized in hydrochloric acid-methanol before extraction of the nicotine. Gas chromatography was carried out on a packed column using polyglycol 4000 as stationary phase on Celite 60-100 mesh at 190°C. From the method, nicotine can be estimated in concentrations of 0.01 µg/ml blood respectively for g tissue by using 5 g of material. The use of greater amounts of material increases the sensitivity.

A method for the determination of submicrogram amounts of nicotine in blood was developed by Burrows et al.³⁶ Samples of 10 ml heparinised blood were used for an analysis, where, after addition of NaOH, the alkaloid was isolated by steam distillation. From solvent partition and column chromatographic clean-up on alumina, a nicotine solution in ethanol was obtained, which was used for the gas chromatographic assay on Carbowax 20 M 8 % columns on Chromosorb W treated with 2 % KOH. Quinoline was used as an internal standard and nicotine down to 1 ng was determined by this method.

The method developed by Burrows et al.³⁶ was modified by Falkman et al.³⁷ to improve the overall sensitivity of the method and to decrease the level of interfering co-extractives. The blood sample was alkalinized with NaOH and steam-distilled, the distillate made acid with sulphuric acid and purified by extraction with dichloromethane, then made alkaline with NaOH and extracted with dichloromethane to isolate the nicotine. The alkaloid was back extracted with sulphuric acid and, after addition of NaOH, extracted with benzene. The benzene solution was used for the gas chromatographic analysis, which was carried out under the same conditions as described by Burrows et al.³⁶. The overall recovery increased from 55 to over 80 % compared with the method of Burrows et al.

In one paper, Isaac and Rand³⁸ described a method for the determination of nicotine in plasma. By using an alkali flame ionization detector the sensitivity of the method was improved to 1 ng/ml of nicotine in a 2.5 ml sample. Modaline was used as an internal standard. The alkaloid was extracted from the basified plasma (NaOH) with diethyl ether, and this extract was used for the gas chromatographic assay on a packed column with 13 % KOH and 6.5 % Carbowax 20 M as stationary phase on Varaport 30.

Due to the poor reproducibility of the method developed by Isaac and Rand³⁸, Feyerabend et al.³⁹ developed a better method for the determination of nicotine in biological fluids. Nicotine was extracted from alkalinized (NaOH) plasma into diethyl ether. This was concentrated by evaporation, and after acid back extraction it was re-extracted into *n*-heptane (N-detector) or dichloromethane (FID) before the gas chromatographic analysis. Quinoline was used as an internal standard. The method is rapid and enables concentrations of 0.1 ng/ml⁻¹ to be measured.

The Isaac and Rand³⁸ direct extraction procedure, improved by Feyerabend et al.³⁹ was again modified by Feyerabend and Russel⁴⁰. The extraction procedure was simplified and the reproducibility of the method improved. The internal standard (quinoline) was added to the samples (3 ml) and these made alkaline and extracted with diethyl ether. The solvent was evaporated

to small bulk and then extracted with dilute acid. An excess of alkali was added and the nicotine extracted with butyl acetate. An aliquot was gas chromatographed on a packed column with 10 % Apiezon and 10 % KOH on Chromosorb at 220°C using a nitrogen-detector. Quantitation relied on the comparison of peak areas and the calibration curve was linear over the concentration range, 0.5 to 100 ng/ml⁻¹. Nicotine concentrations as low as 0.1 ng/ml⁻¹ could be measured.

In addition, a direct micro-extraction technique applied to only 100 µl of sample was developed: An aqueous solution of quinoline (0.075 µg/ml) as internal standard, sodium hydroxide (5M, 400 µl) and di-isopropyl ether (50 µl) were added to samples (100 µl) in a Dreyer tube. After agitation on a Vortex mixer (1 min) the tube was centrifuged (1 min) and 5 µl of the organic layer was injected onto the gas chromatograph. The method yields an accurate result in 5 min.

Hengen and Hengen⁴¹ developed a method for the determination of nicotine and cotinine in 1 ml samples of plasma. Nicotine was extracted from alkalinized plasma (NaOH) with diethyl ether, and cotinine from the same sample with dichloromethane. Modaline was used as internal standard for nicotine, lidocaine for cotinine. Analytical recovery of nicotine added to the plasma was 80 ± 6 %, for cotinine 95 ± 5 %. The internal standards were directly added to the plasma to monitor extraction losses. The sensitivity was such that less than 0.1 µg of nicotine and 0.1 µg of cotinine could be detected per liter. Day-to-day reproducibility for nicotine was within 14 % and within 6 % for cotinine. Narrow peaks for the gas chromatographed compounds were obtained on the very stable SP-2250 column (3 %) on Supelcoport at 155°C for nicotine and 190°C for cotinine.

Pilotti et al.⁴² carried out studies on the identification of tobacco alkaloids, their mammalian metabolites and related compounds by gas chromatography-mass spectrometry using packed columns (SE-30, SE-52 and Carbowax 20 M + KOH) and capillary columns (33 m - Emulphor O and 9.6 m - OV-101). Various pyridine compounds, either identified or implied as intermediates in the mammalian metabolism of nicotine present in tobacco or tobacco smoke, were studied by GC-MS. Preliminary GC-MS experiments on the determination of nicotine using capillary columns in combination with multiple ion detection (MID) employing deuterated nicotine as internal standard were reported. The gas chromatographic data of the compounds investigated are given in Table 5.10.

Dow and Hall⁴³ also used capillary column combined gas chromatography-mass spectrometry. They developed a method for the estimation of nicotine in plasma by selective ion monitoring. A glass capillary coated with SP-1000 was attached directly to a mass spectrometer, which was operated in the SIM (selective ion monitoring) mode. Nicotine could be determined down to 3 ml samples of plasma. The extraction procedure was a modification of the method described by Feyerabend et al.³⁹, which allowed the direct addition of the internal standard (quinoline) to plasma prior to extraction. The calibration curve was constructed by plotting the ratio of the peak heights of the *m/e* 84 ion of nicotine and the *m/e* 129 ion of quinoline against the concentration of nicotine (ng/ml). This plot was linear over the concentration range 5-100 ng nicotine/ml. The method is sensitive and specific without the need for deuterated nicotine as internal standard.

Kogan et al.⁴⁴ described a method for simultaneous determination of nicotine and cotinine in plasma using ketamine as internal standard. After basification of the sample the alkaloids and the added internal standard were extracted with methylene chloride, back-extracted into

acid, and then re-extracted into methylene chloride. Glass columns (1.8 m by 2 mm I.D.) packed with 3 % SE-30 on Gas Chrom Q 100-120 mesh and temperature programming from 150 to 200°C (24°C/min) and nitrogen detection were used. Detector response was linear over a range of 2 to 50 ng/ml nicotine and 50 to 500 ng cotinine

TABLE 5.10

GAS CHROMATOGRAPHIC DATA OF TOBACCO ALKALOIDS AND THEIR MAMMALIAN METABOLITES⁴²

A = Retention times on the SE-30 column (a) = Mixture of R,S- and S,S-dia-
 B = Retention times on the Carbowax (KOH) column stereoisomers
 C = Retention times on the Emulphor-O glass capillary column (b) = Partly decomposed into
 D = Retention times on the SE-52 column nicotine during GLC
 E = Retention times on the OV-101 glass capillary column (c) = Mixture of enantiomers

Compound		Retention times		Mode of recording MS
Ia	S-(-)-Nicotine	6.0/85°C 5.3/135°C 5.0 4.7/142°C	A B C B	GC-MS
Ib	5-d ₂ -S-(-)-Nicotine	4.7/142°C	B	GC-MS
II	S-(=)-Nicotine isomethiodide hydroiodide			Direct probe
III	S-(-)-Nicotine-1'-oxide (a)	10.6	C(b)	Direct probe
IV	S-(-)-Nicotine-1,1'-dioxide (a)			Direct probe
V	S-(-)-Nornicotine	9.0/90°C 10.7	A C	GC-MS
VI	Myosmine	5.9/90°C	A	GC-MS
VII	β-Nicotyrine	5.0/100°C 13.2	A C	GC-MS
VIII	S-(-)-Anabasine	5.6/100°C 10.8	A C	GC-MS
IX	S-(-)-Methylanabasine	4.5/100°C 5.3	A C	GC-MS
X	S-(-)-Cotinine	5.8/130°C 7.0/213°C 23.2 3.2/170°C 5.4	A B C D E	GC-MS
XI	S-(-)-Cotinine methiodide			Direct probe
XII	S-(-)-Cotinine-1-oxide			Direct probe
XIII	S-(-)-Norcotinine	5.9/135°C 10.9/265°C 4.4/170°C	A B D	GC-MS
XIV	(5S,3R)-3-Hydroxycotinine	5.7/140°C 4.6/170°C	A D	GC-MS
XV	Dihydrometanicotine	4.9/105°C 9.9	A C	GC-MS
XVI	trans-Metanicotine	4.3/110°C 13.5	A C	GC-MS
XVII	cis-Metanicotine	3.8/110°C	A	GC-MS
XVIII	4-(3-Pyridyl)butyric acid			Direct probe
XIX	Methyl 4-(3-pyridyl)butyrate	6.8/95°C	A	GC-MS
XX	4-(3-Pyridyl)-4-oxobutyric acid			Direct probe
XXI	Methyl 4-(3-pyridyl)-4-oxobutyrate	4.3/125°C 19.1	A C	GC-MS
XXII	4-(3-Pyridyl)-4-hydroxybutyric acid (c)			Direct probe
XXIII	Methyl 4-(3-pyridyl)-4-hydroxybutyrate	5.9/140°C 19.0	A C	GC-MS
XXIV	5-(3-Pyridyl)tetrahydrofuran-2-one (c)	6.0/140°C 23.2 5.8	A C E	GC-MS
XXV	4-(3-Pyridyl)-4-oxobutyramide	5.7/145°C	A	GC-MS
XXVI	Allohydroxycotinine (c)	5.6/140°C 6.8/213°C 3.0/170°C	A B D	GC-MS

TABLE 5.10 (continued)

	Compound	Retention times		Mode of recording MS
XXVII	3-Pyridylacetic acid	3.3/85°C 7.9	A	Direct probe GC-MS
XXVIII	Methyl 3-pyridylacetate		C	
XXIX	N-(3-pyridylacetyl)glycine	5.7/150°C	A	Direct probe GC-MS
XXX	Methyl N-(3-pyridylacetyl)glycinate			

In order to determine nicotine and cotinine in low nanogram levels in 1 g samples of tissue homogenates, that are more complex biological matrices than biological fluids, Thompson et al.⁴⁵ used 12 m long fused silica capillary columns deactivated with Carbowax 20 M and coated with a dimethyl silicone liquid. Close structural analogues, methylanabasine (for nicotine) and 1'-trideuteromethyl-nornicotine (for cotinine) were used as internal standards. Nicotine and cotinine were extracted separately after addition of NaOH to the homogenate, with ethyl acetate (nicotine) and toluene (cotinine). Nicotine was gas chromatographed by 80-150°C and cotinine by 120-200°C - both 4°C/min. For the nicotine determination a nitrogen-detector was used. Cotinine could only be quantified by chemical ionization GC-MS methods.

Jacob et al.⁴⁶ developed a method for the determination of nicotine and cotinine in plasma and urine samples using structural analogues for both compounds as internal standards, N-ethylnornicotine for nicotine and N-(2-methoxyethyl)-nornicotine for cotinine. Glass columns (1.8 m for nicotine and 1.2 m for cotinine, by 2 mm I.D.) packed with 2 % Carbowax 20 M + 2 % KOH on Gas Chrom P 100-120 mesh or 3 % SP-2250 DB on Supelcoport 100-120 mesh were used, and column temperatures 145°C for nicotine and 210°C for cotinine. The alkaloids were extracted from the sample with diethyl ether (nicotine) or butanol (cotinine) after addition of NaOH, back extraction into acid and re-extraction into diethyl ether (nicotine) or methyl-chloride (cotinine). Linearity in the range of 0-100 ng/ml nicotine and 0-1000 ng/ml cotinine was achieved by means of a nitrogen-detector.

A special application of gas chromatographic determination of nicotine in biological fluids is its determination in the breast fluid of non-lactating women. By using a combination of gas chromatography, mass spectrometry and a selected ion recording technique, Petrakis et al.⁴⁷ identified nicotine and its major metabolite cotinine in the breast fluid of non-lactating women smokers. As little as 25 picograms could be measured by using the deuterated variants, (5',5'-²H)-nicotine and (3,3-²H)-cotinine, both as internal standards and as carriers in an inverse isotope dilution method.

5.1.5. Miscellaneous

5.1.5.1. Nicotine in residues on foods

Martin⁴⁸ used gas chromatography to determine nicotine residues on mustard green samples. Samples of mustard green were analysed by the US official method, modified by the author for gas chromatography on a packed DC-200 column 10 % on Gas Chrom Q. The identity of the nicotine peak was confirmed by TLC after trapping the eluted compound from the gas chromatographic column. Recovery obtained on five samples spiked at the 1-3 ppm level ranged from 95 to 97 %.

5.1.5.2. *Biosynthesis of nicotine alkaloids*

Almost the same method as described by Quin and Pappas⁴ was used by Alworth et al.⁴⁹ for investigations on the biosynthesis of nicotine in *Nicotiana glutinosa*. The assay of nicotine was carried out on the aerial and root sections of the plant separately. Polybutylene glycol 10 % on Firebrick was used, at column temperature 169°C.

For studies on the interrelationship among nicotine, nornicotine, anabasine and anatabine during the biosynthesis in *Nicotiana glutinosa*, Alworth and Rapoport⁵⁰ used almost the same gas chromatographic conditions as reported above. On a polybutylene glycol column the alkaloids mentioned were satisfactorily resolved.

5.1.5.3. *Airborne nicotine*

A method for the determination of airborne nicotine was developed by Crouse et al.⁵¹. The air was taken up in water by suction of the air through a tube with water. The solution obtained in that way was gas chromatographed directly on a 58 inch. x 3 mm O.D. glass column packed with 5 % polyphenyl ether (6 ring), on Anakrom ABS 110-120 mesh at 190°C using FID. The minor airborne tobacco alkaloids, nornicotine, myosmine and anabasine, did not interfere with nicotine. The standard curve was linear over a range of 10-500 µg/ml with a relative standard deviation of 95 % for 10-50 µg/ml.

TABLE 5.11

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF TOBACCO ALKALOIDS

Column	Solid support mesh	Stat.phase	%	Temperature	Comp.Preparation	Ref.
glass, 1 m x 6 mm O.D.	Fib. BW	PEG 20M	25	190°C	s.to.alk.	1
	Fib. BW	PPG 1025	25	190°C		
	Fib. BW	PBG 1500	25	180°C		
glass, 1 m x 6 mm O.D.	Fib. BW	PPG 1025	25	145°C	s.alk.to.sm.	2
	Fib. BW	PBG 1500	25	190°C		
	Fib. BW	PEG 20 M	25	190°C		
	Fib. BW	PEG 4000	25	190°C		
glass, 1 m x 10 mm O.D.	Fib. BW	PPG 1025	25	190°C		
glass, 1 m x 6 mm O.D.	Fib. BW	PPG 1025	25	190°C	s.alk.to.sm.	3
	Fib. BW	PEG 20 M	25	190°C		
glass, 1 m x 10 mm O.D.	Fib. BW	PPG 1025	25	190°C		
glass, 1 m x 6 mm O.D.	Fib. BW 30-60	PPG 1025	25	190°C	ni.qnt.to. noni.qnt.to.	4
	Fib. BW	PBG 1500	25	180°C		
glass, 2 m x 6 mm O.D.	Fib. BW 30-60	PEG 20 M	25	180°C	noni.my.s.	
glass, 1 m x 14 mm O.D.	Fib.	PPG 1025	25	180°C	ni.noni.pre.	
no inf.		Sil.gr. + KOH PEG 6000 + KOH			alk.s.	5
		PEG 1500 + KOH		140°C	alk.qnt.	6
		PEG 6000 + KOH		210°C		
glass, 2 m x 5 mm	Fib.	PEG 20 M	5.6	200°C	alk.s.	7
glass, 2.44 m x 3.5 mm	CW AWS 60-80	SE-30	5	190°C	alk.s.	8
	I.D. CW AWS 60-80	Ver.	10	170°C		

TABLE 5.11 (continued)

Column	Solid support	Stat.phase	%	Temperature	Comp.Preparation	Ref.
glass, 2.44 m x 3.5 mm I.D.	CW AWS 60-80	DC 550	10	170°C	alk.s.	8
cop., 6 ft x 1/8 in O.D.	GP 100-120	JXR	1	100-300°C pr.		
	Dia S 80-100	SE-52	1	100-300°C pr.		
	Dia S 80-100	XE-60	1	100-250°C pr.	alk.s.	9
s.s. cap. 100 ft x 0.01 in I.D.		QF-1		100-200°C pr.		
s.s. cap. 200 ft x 0.01 in I.D.		SE-30		100-250°C pr.		
s.s. cap. 100 ft x 0.01 in I.D.		Apiezon L		100-250°C pr.		
s.s. cap. 50 m x 0.5 mm		Ucon LB 550 X + KOH		195°C	alk.s.	10
glass, 2 m x 2 mm I.D.	CG 100-120	OV-17	1.25	136°C (FID) 185°C (ECD)	ni.qnt.der.	11
glass, 0.9 m x 2 mm I.D.	CW 80-100	OV-1	3	210°C		
		+ OV-17	3			
glass, 2.1 m x 2 mm I.D.	GZ 80-100	OV-17	3	225°C		
glass, 1.2 m x 2 mm I.D.	GQ 80-100	OV-17	3		ni.qnt.der.	12
		+ Cab 20 M	0.6	200°C		
		+ terphth.a.				
glass cap. 10 m x 0.77 mm I.D.		SE-30		200°C		13
no inf.	Cel 545	PEG 20 M	25	200-220°C	ni.qnt.	14
glass, 3.05 m x 2 mm I.D.	CW AWS 60-80	DC 550	10	185°C	alk.qnt.to.	15
s.s., 3 ft x 1/8 in	CW 60-80	Castor	10	160°C	ni.qnt.to.	16
		+ KOH	3			
glass, 4 m x 3 mm I.D.	Diat M 80-100	Cab 20 M	6	180°C	alk.qnt.to.	17
		+ KOH	1			
no inf. 6 ft	Sup 80-100	Cab 20 M	7	180-200°C pr.	alk.qnt.to.	
		+ Ppe 6	3	1°C/min		
		+ KOH	2			18
no inf. 3 ft	Sup 80-100	Cab 20 M	7	180-200°C pr.	pyr.to.alk.	
		+ Ppe 6	3	1°C/min		
		+ KOH	2			
glass cap. 35 m x 0.25 mm I.D.		Cab		170-200°C pr. 2°C/min		
glass cap. 15 m x 0.25 mm I.D.		Superox TM -4		130-200°C pr. 4°C/min	to.alk. qnt.	19
glass, 6 ft x 1/8 in	GQ 80-100	NGA	10	168°C	ni.qnt.to.sm.	20
no inf.	CW	Apiezon L	20		alk.to.sm.	21
no inf.	Cel 545	PEG 20 M	25	190°C	ni.qnt.to.sm.	22
glass, 1.5 m x 3 mm	Dias L 60-80	Castor	5	160°C	ni.qnt.to.sm.	23
glass, 2 m x 3 mm	Dias L 60-80	Castor	5	150°C	ni.qnt.to.sm.	24
s.s., 6 ft x 1/8 in	GQ 80-100	Cab 20 M	7	170-190°C	ni.qnt.spm.	25
		+ Ppe 6	3			
		+ KOH	2			
glass, 1.8 m x 2 mm I.D.	CW HP	Cab 20 M	10	no inf.	ni.to.sm.	26
glass, 12 ft x 3-4 mm I.D.						
s.s., 1 m x 1/8 in O.D.	Ana ABS 100-110	SE-30	14.5	200°C	ni.qnt.ur.	27
	Dia S 80-100	Cab 20 M	2	135°C	ni.qnt.ur.	28,29
		+ KOH	5	209°C	cot.qnt.ur.	
s.s., 1.5 m 1/8 in	Fib. C 22 AW	Apiezon	8	185°C		
		+ KOH	4			
s.s., 1.5 m x 1/8 in	Fib. C 22 AW	Ver	5	90°C		
		+ KOH	2		ni.qnt.ur.	30
s.s., 2.1 m x 1/8 in	CW AWS	Ver	5	130°C		
		+ KOH	2			
s.s., 1.5 m x 1/8 in	GQ	Uc.HB Po	3.2	120°C	ni.qnt.ur.	31
		+ KOH	6			
s.s., 1.5 m x 1/8 in O.D.	GQ	Uc.HB Po	3.2	120°C	ni.cot.qnt.ur.	32
		+ KOH	6			

TABLE 5.11 (continued)

Column	Solid support	Stat. phase	%	Temperature	Comp.Prep.	Ref.
s.s., 1 m x 1/8 in O.D.	GQ 100-120	Uc.HB Po	3.2	110-180°C pr.	ni.cot.qnt.	33
glass cap. 20-28 m x 0.32-0.34 mm I.D.		+ KOH	6	20°C/min	pl. ur.	
		SE-52		40°C pr.	30°C/min	34
glass, 1.8 m x 1/4 in	Cel 60-100	PEG 4000	8	80°C pr.	10°C/min	
				230°C	ni.bl.tis.	35
glass, 2 m x 4 mm I.D.	CW AWS 80-100	Cab 20 M	8	150°C	ni.qnt.bl.	36
glass, 2 m x 3 mm I.D.	CW AWS 80-100	+ KOH	2	150°C	ni.qnt.bl.	37
		Cab 20 M	8			
s.s., 5 ft x 1/8 in	Var 30 80-100	+ KOH	2	146°C	ni.qnt.pl.	38
glass S, 6 ft x 6 mm	CW 80-100	Cab 20 M	6.5			
		+ KOH	13	170°C	ni.qnt.bifl.	39
glass S, 6 ft x 6 mm	CW 80-100	Apiezon L	10			
		+ KOH	10	220°C	ni.qnt.bifl.	40
glass, 1.8 m x 2 mm I.D.	Sup 100-120	Apiezon L	10			
		+ KOH	10	155°C	ni.qnt.pl.	41
glass, 1.7 m x 1 mm I.D.	GQ 100-120	SP-2250-DB	3			
				190°C	cot.qnt.pl.	
cap. 33 m x 0.4 mm I.D.	GQ 100-120	Cab 20 M	8	142°C		
		+ KOH	2			
cap. 9.6 m x 0.2 mm	GQ 100-120	SE-30	3		alk. + met.	42
		+ KOH	2			
glass cap. 20 m x 0.3 mm I.D.		SE-52	5		pl. MS	
		+ KOH	2			
cap. 33 m x 0.4 mm I.D.	GQ 100-120	Emulphor 0		125-200°C pr.		
		OV-101				
glass cap. 20 m x 0.3 mm I.D.		SP-1000		160°C	ni.qnt.pl.	43
glass, 1.8 m x 2 mm I.D.	GQ 100-120	SE-30	3	150-200°C pr.	ni.cot.qnt.	44
f.sil. cap. 12 m x 0.2 mm I.D.		Dim.sil.liqu.		24°C/min	pl.	
glass, 1.8 m x 2 mm I.D.	GP 100-120	SE-30	2	80-150°C pr.	ni.	qnt.tis.45
glass, 1.2 m x 2 mm I.D.	GP 100-120	+ KOH	2	140°C	ni.	qnt.pl. 46
		SP 2250 DB	3			
glass, 2 m x 2 mm		+ KOH	2	210°C	cot.	
		Cab 20 M	2			
glass, 6 ft x 4 mm I.D.	GQ	+ KOH	2	140°C pr.	ni.qnt.brfl.	47
		DC-200	10			
s.s., 5 ft x 0.5 in	Fib. 60-80	PBG	10	5°C/min	MS	
		+ KOH	10			
s.s., 5 ft x 1/4 in	Fib. 60-80	PBG	10	125°C	ni.res.food	48
		+ KOH	10			
glass, 50 in x 3 mm O.D.	Ana ABS 110-120	PBG	10	169°C	alk. biosynth.	50
		+ KOH	10			
glass, 50 in x 3 mm O.D.	Ana ABS 110-120	Ppe 6	5	180-200°C	alk. air.	51

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TABLE 5.12

TOBACCO ALKALOIDS - LIST OF ABBREVIATIONS

ABS = acid, base washed, silanized	met = metabolite
alk = alkaloid	MF = mass fragmentography
Ana = Anakrom	MD = mass spectrometry
anab = anabasine	my = myosmine
AW = acid washed	ni = nicotine
bi = biological material	no inf. = no information
bifl = biological fluid	noni = nornicotine
bl = blood	ND = nitrogen detector
brfl = breast fluid	NGA = neopentyl adipate
BW = base washed	O.D. = outside diameter
Cab = Carbowax	PBG = polybutylene glycol
cap = capillary	PEG = polyethylene glycol
Castor = Castorwax	pl = plasma
Cel = Celite	PPG = polypropylene glycol
CG = Chromosorb G	Ppe6 = polyphenylether (6-ring)
cif = cigarette filter	pre = preparative
comp = compound	pur = purification
cop = copper	pyr = pyrolysis
cot = cotinine	qnt = quantitative
der = derivative	s = separation
Dia S = Diatoport	S = silanized
Dias L = Diasolid L	sil.gr. = silicone grease
Diat = Diatomite	SIM = selective ion monitoring
Dim.sil.liqu. = Dimethylsilicone liquid	sm = smoke
ECD = electron capture detector	spm = smoke particulate matter
Fib = firebrick	s.s = stainless steel
f.sil = fused silica	Sup = Supelcoport
GP = Gas Chrom P	terpht = terephthalic acid
GQ = Gas Chrom Q	tis = tissue
GZ = Gas Chrom Z	to = tobacco
HP = high performance	ur = urine
I.D. = inner diameter	Uc HB Po = Ucon HB 2000 Polar
id = identification	Var = Varaport
in = inch	Ver = Versamid 900
is = internal standard	
JXR = dimethylpolysiloxane	

Chapter 6

PIPERIDINE ALKALOIDS

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6.1 *PIPER NIGRUM* ALKALOIDS

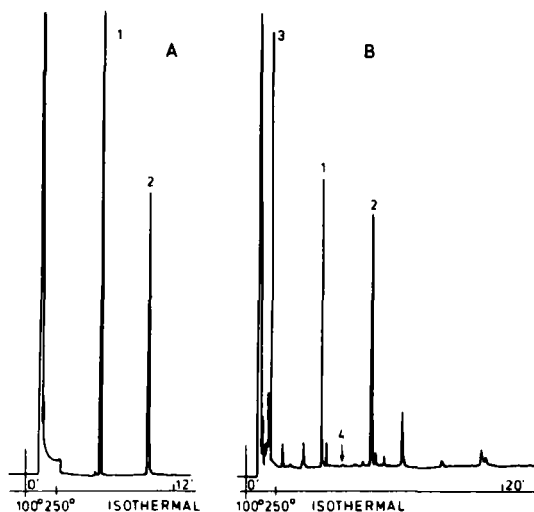
Piperine (1-piperoylpiperidine), alkaloid from *Piper nigrum* L., belonged to the group of alkaloids which, in 1960, was gas chromatographed on a packed SE-30 column by Lloyd et al.¹.

Capillary gas chromatography and cold on-column injection was used by Verzele et al.² for quantitative determination of piperine in pepper and pepper extract. Piperine was extracted from samples of 70 mg ground pepper or 10 mg pepper extract with dichloromethane containing the internal standard, tetrahydropiperine, and the dichloromethane extract obtained was used for gas chromatography. A 25 m by 0.5 mm I.D. glass capillary column, deactivated by high-temperature silylation and coated with OV-1, was used. The samples were injected at an oven temperature of 100°C, and the temperature was immediately increased to 250°C. Chromatography of pure piperine (0.435-0.027 mg) against a constant amount of the internal standard gave a straight-line calibration graph passing through the origin. Typical gas chromatograms are shown in Figure 6.1. Good results were obtained, with a standard deviation for a pepper sample ($n = 5$) of 1.03 %.

FIGURE 6.1

GLASS CAPILLARY GC ANALYSIS WITH COLD ON-COLUMN INJECTION OF PIPERINE²

A = pure piperine and B = pepper extract. Internal standard (1) tetrahydropiperine, and piperine (2). Column: 25 m x 0.5 mm I.D. HTS-OV-1 column, 250°C isothermal.



6.2 *CONIUM MACULATUM* ALKALOIDS

Moll³ gas chromatographed some Conium alkaloids and a number of other piperidine bases. A good separation was obtained on a packed column of 30 % polyethylene glycol 4000 on silica gel impregnated with 20 % potassium hydroxide. Potassium hydroxide was used to reduce adsorption to the solid support. The results are given in Table 6.1.

TABLE 6.1

RETENTION TIMES OF SOME PIPERIDINE BASES³

1.8 m long packed column (Silica gel, 20 % KOH + 30 % PEG 4000)

Compound	130°C	160°C	Compound	130°C	160°C
Piperidine	6.75	2.60	2,6-Dimethylpiperidine	5.55	2.25
Morpholine	17.25	4.75	γ-Coniceine	26.50	7.25
Coniine	15.25	4.07	N-Methylconiine	13.60	4.00
Conhydrine	-	28.00	N-Methylconiceine	84.00	16.60

Fairbairn et al.⁴ applied gas chromatography to quantitative assay of the alkaloids in *Conium maculatum* L. The difficulty of preparing a concentrated solution of the volatile bases, without loss, was overcome by extracting the bases in the form of their non-volatile salts and liberating them *in situ* on the column. This was achieved by inserting a small amount of soda lime at the beginning of the column and using a solution of the alkaloids to be analysed in 70 % methanol. Samples of 25-30 g of fresh fruits (= 25-100 mg alkaloid) were basified and extracted with diethyl ether. The diethyl ether layer was extracted with N HCl, and the aqueous layer basified and extracted with diethyl ether again. After addition of 0.5 ml concentrated hydrochloric acid the diethyl ether was evaporated. When the excess of hydrochloric acid had also disappeared, the residue was dissolved in 1 ml 70 % methanol and gas chromatographed. A packed column with 25 % silicone elastomer 301 on Celite was used at a column temperature of 110°C. The separation obtained was sufficient for quantitative assay of the main alkaloids, coniine, M-methylconiine and γ-coniceine. The values obtained by gas chromatography were in good agreement with those obtained by spectrophotometry.

TABLE 6.2

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF PIPERIDINE ALKALOIDS

Column	Solid support mesh	Stat.phase	%	Temperature	Comp. Prep.	Ref.
glass, 6 ft x 4 mm I.D.	CW 80-100	SE-30	2-3	204°C	alk. s.	1
glass cap. S, 25 m x 0.5 mm I.D.		OV-1		250°C	pip.qnt.pm.	2
glass, 1.8 m x 4 mm I.D.	Sig 0.2-0.3 mm	PEG 4000	30	130°C	con.alk.	3
		+ KOH	20			
glass, 6 ft	Cel 70-80	Si.el.	25	110°C	con.alk.	4

Abbreviations: Cel = Celite, con = Conium, CW = Chromosorb W, pip = piperine, S = silanized, Si.el = silicone elastomer, Sig = silica gel.

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Chapter 7

QUINOLIZIDINE ALKALOIDS

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7.1 LUPINE ALKALOIDS

Ten of the 45 alkaloids that were gas chromatographed by Lloyd et al. in 1961¹ on a 2-3 % SE-30 on Chromosorb W column were lupine alkaloids. The bicyclic lupinine and the tricyclic sparteine, α -isosparteine and 13-hydroxysparteine were chromatographed at a column temperature of 160°C, the tricyclic cytisine, methylcytisine, methylcytisine-N-oxide and the tetracyclic lupanine, 13-hydroxylupanine and matrine at 204°C. The retention times of the alkaloids are listed in Table 7.1.

TABLE 7.1

RETENTION TIMES OF LUPINE ALKALOIDS¹

6 ft long packed column (Chromosorb W, 2-3 % SE-30 at 160°C (a) and 204°C (b))

Cytisine	5.1 (b)
Methylcytisine	4.3 (b)
Methylcytisine-N-oxide	5.8 (b)
Lupanine	5.5 (b)
13-Hydroxylupanine	11.6 (b)
Matrine	8.5 (b)
Lupinine	1.5 (a)
Sparteine	5.9 (a)
α -Isosparteine	5.2 (a)
13-Hydroxysparteine	14.3 (a)

Faugeras and Paris² applied gas chromatography to an investigation of the alkaloids in *Genista pilosa* L. fruits. The alkaloids were extracted with alcohol containing tartaric acid and water containing 5 % sulphuric acid. After purification of the aqueous acidic extracts by extraction with diethyl ether and chloroform, the alkaloid bases were extracted with chloroform after adjustment of the pH to > 12. Gas chromatography demonstrated the presence of three alkaloids, two of which were identified, by means of their retention times on a 10 % SE-30 column on Chromosorb W, as cytisine and N-methylcytisine (retention times 6.2 min and 5.7 min respectively on a 3 m long column at 245°C).

In another paper, Faugeras and Paris³ investigated the alkaloids in *Genista acanthoclada* DC. The plant material was stabilized in boiling methanol and completely extracted with the same solvent. After evaporation of the solvent, the residue was taken up in water and the aqueous solution extracted with diethyl ether after addition of hydrochloric acid. After adjustment of the pH to 10 the alkaloid bases were extracted with diethyl ether and the extract obtained used for the gas chromatographic analysis, which was performed with packed columns using SE-30 and SE-52 as stationary phases. The retention times of the alkaloids found are given in Table 7.2.

TABLE 7.2

RETENTION TIMES OF ALKALOIDS IN *GENISTA ACANTHOCLADA* DC³

1.5 m long packed column (Aeropak, 5 % SE-30 and Chromosorb W, 5 % SE-52) at 205°C

	SE-30	SE-52
Retamine	1.3	3.7
Cytisine	3.0	5.5
Anagryne	7.0	10.0
N-methyl cytisine		

Cranmer and Mabry⁴ applied gas chromatography to investigate the alkaloids in sixteen species belonging to the genus *Baptisia*. The alkaloids were extracted with methylene chloride made basic with ammonium hydroxide. After concentration of the solution the alkaloids were taken up in aqueous citric acid solution and this was extracted with methylene chloride to remove impurities. The pH of the aqueous solution was then adjusted to 10 with ammonium hydroxide and the alkaloid bases extracted with methylene chloride. After concentration of the methylene chloride extract it was gas chromatographed on a packed column of 3 % XE-60 and 5 % DC 550 on Chromosorb W at 220-223°C. The retention times are given in Table 7.3.

TABLE 7.3

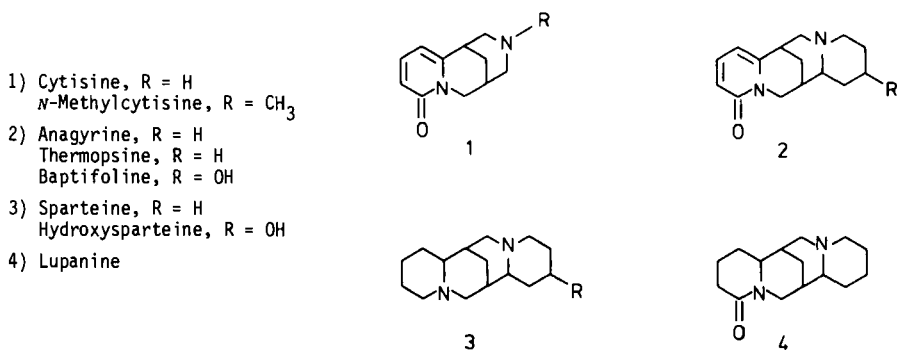
RETENTION TIMES OF SOME LUPINE ALKALOIDS⁴

1.3 m long packed column (Chromosorb W, 3 % XE-60 and 5 % DC-550) at 220-223°C

Alkaloid	XE-60	DC-550
Anagryne	12.1	87.0
Cytisine	4.90	27.0
Methylcytisine	2.80	20.5
Lupanine	2.60	26.4
Hydroxylupanine	17.4	c.200
Lupinine	0.50	c.2.0
Sparteine	1.00	4.6
13-Hydroxysparteine	1.40	12.3
17-Oxysparteine	1.65	14.1
Thermopsine	9.70	54.0
Baptifoline	c.30	275.0

FIGURE 7.1

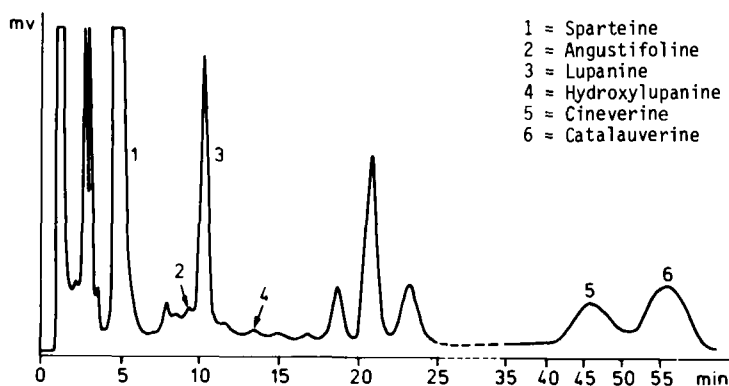
CHEMICAL STRUCTURES OF SOME LUPINE ALKALOIDS



Faugeras and Paris⁵ carried out an investigation of the alkaloids of *Sarothamnus catalunicus* Webb. by means of gas chromatography. The alkaloids were extracted in a Soxhlet apparatus with methanol, then with methanol containing 0.15 % tartaric acid. After addition of water the methanol was evaporated and the acidic aqueous solution extracted with diethyl ether to remove impurities. Sodium carbonate was added to obtain pH 9 and the alkaloid bases were extracted with diethyl ether and chloroform. The diethyl ether-chloroform extract was concentrated and gas chromatographed. Because of the slightly volatile cineverine and catalauverine, a 30 cm long packed column with 10 % SE-52 and temperature programming from 190°C to 285°C was used for the gas chromatographic analysis. A gas chromatogram of the alkaloids is shown in Figure 7.2.

FIGURE 7.2

GAS CHROMATOGRAM OF ALKALOIDS IN *SAROTHAMNUS CATALAUNICUS*⁵
30 cm long column (Aeropak 30, 10 % SE-52), temperature programming 190-285°C



Gas chromatographic separation and isolation of microgram amounts of lupine alkaloids combined with mass spectrometry was used by Cho and Martin⁶ for the unambiguous identification of 20 such alkaloids. The retention times on a packed column of 10 % QF-1 and the five most abundant ions by spectrometry are listed in Table 7.4.

Owing to the value of lupine seeds as a protein source and the toxicity of the alkaloids that may be present even in "sweet" lupine seeds, Ruiz⁷ developed a gas chromatographic method to analyse the alkaloids in such seeds. The sample (2 g seed) was finely ground and extracted with ammoniacal chloroform, and the alkaloids removed from the chloroform with a 0.1 N sulphuric acid. After basification the alkaloid bases were extracted with chloroform. The internal standard (caffeine) was added and the solvent evaporated. The residue was dissolved in ethanol and the solution obtained was gas chromatographed on a 8 % JXR packed column on Chromosorb W. The retention times of the alkaloids are listed in Table 7.5.

The alkaloids of the overground parts of *Lupinus argenteus* Pursh. var. *stenophyllus* (Rydb.) Davis were investigated using gas chromatography by Keller and Zelenski⁸. The pres-

TABLE 7.4

RETENTION TIMES AND THE FIVE MOST ABUNDANT IONS OF SOME LUPINE ALKALOIDS BY MS⁶
 1.3 m long packed column (Chromosorb W, 10 % QF-1)

Alkaloid	M+	Five most abundant ions					t _R (min)
Δ^{11} -Dehydrolupanine	246	246	134	148	245	247	46.1
Sparteine	234	137	234	98	193	136	7.0
13-Hydroxylupanine	264	152	264	246	55	165	64.0
Lupinine	169	83	152	138	169	97	4.4
Cytisine	190	190	146	147	134	160	42.2
Angustifoline	234	193	112	194	55	150	41.3
N-Methylcytisine	204	58	204	146	160	205	40.2
4-Hydroxylupanine (nuttaline)	264	264	136	134	263	150	44.1
Anagryne	244	98	244	97	146	243	59.5
Lamprolobine	264	138	264	83	97	55	39.4
Lupanine	248	136	248	149	150	247	41.5
Thermopsine	244	98	244	97	146	96	53.5
Isosoporphamine	244	244	243	149	245	148	54.5
α -Isolupanine	248	136	248	149	247	98	40.0
17-Oxolupanine	262	262	150	234	110	263	67.0
Δ^5 -Dehydrolupanine	246	98	97	246	245	121	36.2
Matrine	248	248	96	247	150	137	46.2
17-Oxosparteine	248	97	248	98	110	223	35.6
Rhombifoline	244	58	98	55	146	160	46.1
8-Ketosparteine	248	98	150	97	151	55	32.4

TABLE 7.5

RETENTION TIMES OF LUPINE ALKALOIDS⁷

2 m long packed column (Chromosorb W, 8 % JXR) 140-240°C temperature programming

Alkaloid	t _R (rel)
Lupinine	0.371
Cytisine	1.468
Sparteine	1.611
Lupanine	1.800
Anagryne	2.384
Caffeine (int.stand.) 1	→ t _R = 11.31 min

ence of sparteine, β -isoparteine, Δ^5 -dehydrolupanine, α -isolupanine, lupanine, thermopsine and anagryne was suggested by gas chromatography. Gas chromatography-mass spectrometry confirmed these findings. On a 3 % OV-17 column on Gas Chrom Q, sparteine and genisteine could not be separated. The retention times relative to sparteine were:

Sparteine	1.00
β -Isoparteine	1.29
Δ^5 -Dehydrolupanine	3.20
α -Isolupanine	3.36
Lupanine	3.76
Thermopsine	5.06
Anagryne	5.61

The alkaloids were extracted from air-dried plant material with ethanol, the ethanol extract concentrated, acidified with acetic acid and extracted successively with diethyl ether, ethyl acetate and chloroform to remove impurities. The acidic aqueous solution was made alkaline with ammonia and extracted with chloroform. After concentration this chloroform extract was gas chromatographed on a packed 3 % OV-17 column on Gas Chrom Q by temperature

programming. Combined GLC-MS was carried out on a 2 m long by 2 mm I.D. glass column packed with 3 % OV-17 on Gas Chrom Q and temperature programming, 160°C to 265°C at 2°C/min.

Daily et al.⁹ investigated the content of alkaloids in the Bulgarian *Chamaecytisus* species qualitatively by means of gas chromatography. The dried ground plant material was extracted with methanol and the methanol extract concentrated. After addition of hydrochloric acid, neutral and acidic compounds were removed by extraction with ethylene chloride. The aqueous phase was made alkaline with sodium carbonate and the alkaloid bases extracted with ethylene chloride. After removal of the solvent the residue was dissolved in methanol and analysed by gas chromatography on a packed column of 1.5 % SE-30 on Chromosorb W using temperature programming from 150°C to 250°C.

TABLE 7.6

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF LUPINE ALKALOIDS

Column	Solid support mesh	Stat. phase	%	Temperature	Comp. Prep.	Ref.
glass, 6 ft x 4 mm I.D.	CW 80-100	SE-30	2-3	160°C and 204°C	alk.s.	1
s.s. 3 m x 3 mm I.D.	CW 60-80	SE-30	10	245°C	alk.pm.	2
s.s. 1.5 m	CW	SE-52	5	205°C	alk.pm.	3
s.s. 1.5 m	Aer	SE-30	5	205°C		
s.s. 6 ft x 0.25 in	CW AWS	XE-60	3	220-223°C	alk.pm.	4
s.s. 6 ft x 0.25 in	CW AWS	DC 550	5			
s.s. 30 cm	Aer	SE-52	10	190-285°C pr. 4°C/min	alk.pm.	5
glass, 1.3 m x 4 mm	CW AWS	QF-1	10	230°C	alk.pm.	6
glass, 2 m x 1.6 mm I.D.	CW 100-120	JXR	8	170-240°C pr. 4°C/min	alk.pm.	7
glass, 2 m x 2 mm I.D.	GQ	OV-17	3	125-265°C pr. 4°C/min	alk.pm.	8
2 m x 3 mm	CWS 100-200	SE-30	1.5	150-250°C 20°C/min	alk.pm.	9

Abbreviations: Aer = Aeropak 30, alk = alkaloid, CW = Chromosorb W, GQ = Gas Chrom Q, pm = plant material, S = silanized, s = separation, s.s = stainless steel.

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II.2. TROPANE ALKALOIDS

Chapter 8

TROPINE ALKALOIDS

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8.1. SOLANACEAE ALKALOIDS

Atropine belongs to the first group of alkaloids that was gas chromatographed by Lloyd et al.¹ using packed columns with a support thinly coated (2-3 %) with a non-polar, thermo-stable stationary phase (SE-30). Brochmann-Hanssen and Baerheim Svendsen² in their studies on gas chromatography of alkaloids and alkaloidal salts, used a solid support which was acid and base washed, silanized (hexamethyldisilazane) and treated with 0.1 % polyethylene glycol prior to coating with the stationary phase (SE-30) 1.15 % in order to prevent tailing of the alkaloids during the gas chromatography (due to adsorptive effects of the support material). On a 2 m long glass column by 3 mm I.D., they obtained a good separation of atropine and scopolamine. From gas chromatography of extracts of crude drugs (*Belladonna*, *Stramonium* and *Hyoscyamus*) the ratio hyoscyamine/scopolamine could be roughly estimated.

Atropine and scopolamine were, under certain experimental conditions, dehydrated to their apo-compounds, giving two peaks on the chromatogram: atropine/apoatropine and scopolamine/aposcolamine. The degree of dehydration was found to be associated with the amount of glass wool placed on the top of the column packing and with the temperature of the injection port. When a fairly large amount of glass wool was used, the degree of decomposition decreased as the injection port temperature was reduced, but could never be entirely eliminated. When the amount of glass wool was reduced to a very small amount or removed completely, no decomposition took place even at injection port temperatures of 350°C.

Vigneron and Pelt³ applied gas chromatography for investigations of the alkaloids present in some *Solanaceae* plants. With a 5 % SE-30 column they observed that some dehydration of atropine and scopolamine took place, but interpreted this dehydration as being connected with the solvent used for the extraction and gas chromatography of the alkaloids. When ethanol was used, more apo-alkaloids were found than when chloroform was used. From gas chromatography of a three-month-old solution of atropine in ethanol, a relatively large amount of apoatropine was observed on the chromatogram.

Achari and Newcombe⁴ studied the influence of the tubing material (glass - stainless steel) after silanization of the inside of the tubing. The columns were packed with XE-60 (10 %) and with a mixture of XE-60 (2.5 %) and QF-1 (2.5 %) on Chromosorb W 80-100 mesh (HMDS). On the 10 % XE-60 stainless steel column, scopolamine and hyoscyamine were not eluted at all - as will be seen from Table 8.1.

In a paper on gas chromatographic screening of toxicological extracts for alkaloids, Parker et al.⁵ separated hyoscyamine (atropine), scopolamine and homatropine on packed columns of stainless steel without any problems, as mentioned by Achari and Newcombe⁴. They used

TABLE 8.1

COMPARISON OF THE EFFICIENCIES OF GLASS AND STEEL COLUMNS⁴

x = Compound not eluted; Conditions: Column length 152 cm, I.D. 5 mm; support Chromosorb W 80-100 mesh (HMDS); oven temp. 200°C; injection heater temp. 220°C; carrier gas flow 50 ml/min.

Compound	Retention time (sec)			
	10 % XE-60		2.5 % QF-1 + 2.5 % XE-60	
	glass	steel	glass	steel
Tropine	10	21	5	26
3,6-Dihydroxytropine	38	85	14	78
3-Tigloyloxytropine	28	59	14	73
Scopolamine	269	x	222	558
Hyoscyamine	99	x	165	302
3,4-Ditigloyloxytropine	250	524	222	48

SE-30 (5 %) and Carbowax 20 m (1 %) as stationary phases on Chromosorb 60-80 mesh, acid washed. A solid injector was employed to prevent contamination of the column by non-volatile compounds present in the extracts of the biological material investigated. Satisfactory separation of the tropane alkaloids mentioned was obtained at temperatures above 210°C on the SE-30 column.

Although a satisfactory separation of atropine and scopolamine can be achieved on a non-polar stationary phase (SE-30), a better separation can be obtained on more polar stationary phases, as shown by Brochmann-Hanssen and Fontan⁷, see Table 8.2.

TABLE 8.2

RELATIVE RETENTION VALUES OF SOME TROPANE AND RELATED ALKALOIDS ON STATIONARY PHASES OF VARIOUS POLARITY⁷

Compound	SE-30 1 %		XE-60 1 %	EGSS-Y 1 %	HI-EFF-8B 1 %	
	195 ⁰	225 ⁰	220 ⁰	230 ⁰	230 ⁰	240 ⁰
Atropine	0.51	-	0.59	0.45	0.46	0.46
Scopolamine	0.84	-	1.40	1.13	1.10	1.12
Homatropine	0.31	-	0.39	0.29	0.28	0.30
Cocaine	0.52	-	0.48	0.36	0.31	0.32
Codeine	1.00	1.00	1.00	1.00	1.00	1.00
Codeine time (min)	16.4	1.7	3.6	5.0	8.7	5.6

Also, Moffat et al.⁸ studied the separation of a number of basic drugs, including atropine, on stationary phases of different polarity. They concluded that a low polarity phase, such as SE-30 or OV-17, should be chosen as the "preferred" liquid phase for the GLC of drugs.

The efficiencies of different stationary phases on the separation of some natural tropane compounds and four closely related diesters of 3,6-dihydroxytropine (ditigloyl, disenecioyl, diisovaleryl and di-2-methylbutyl) was also studied by Achari and Newcombe⁴. The results obtained are given in Table 8.3

In Figure 8.1 a chromatogram is given of the separation of some tropanes.

The technique was applied to the analysis of various minor alkaloids in samples of the roots of two species of *Datura* after fractionation of an alkaloidal extract on a kieselguhr

TABLE 8.3

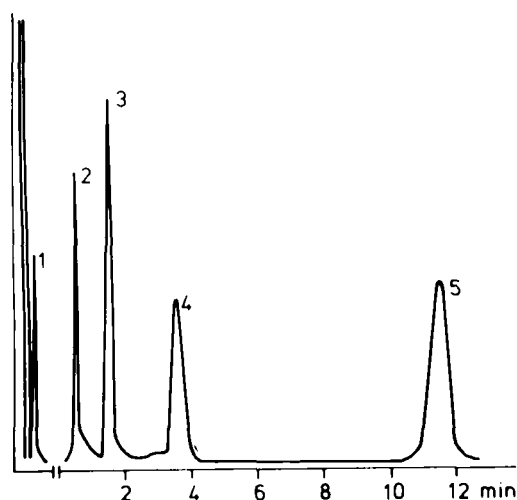
COMPARISON OF THE EFFICIENCIES OF DIFFERENT STATIONARY PHASES⁴

Column 137 cm long, I.D. 4 mm (glass); support Chromosorb W 80-100 mesh (HMDS); oven temp. 180° (a 200°, b 215°, c 250°); carrier gas flow 50 ml/min.

Compound	Retention time (sec)				
	SE-30 2 %	XE-60 2 %	Versamid ^c 5 %	QF-1 2.5 %	PEGS 2.5 %
Tropine/pseudotropine	12	7	19	14.1	28
3,6-Dihydroxytropine	38	24	47	59	76
3-Tigloyloxytropine	62	26	38	57	165
3,6-Ditigloyloxytropine	250	272	165	560 220 ^a	1143
3,6-Diseneciolyoxytropine	241	269	161	567 208 ^b	1152
3,6-Diisovaleryloxytropine	123	95	66	90	1068
3,6-Di-(2-methylbutyryl)-oxytropine	113	90	62	87	1039
Scopolamine	236	274	189	695 217 ^b	1596
Hyoscyamine	180	109	118	165	965

column prior to the gas chromatographic determination of the alkaloids. The analytical results achieved were in good agreement with results obtained by partition chromatographic analysis of the scopolamine and hyoscyamine content in the aerial parts of *Datura stramonium* and *Atropa belladonna* (Table 8.4).

FIGURE 8.1

GAS CHROMATOGRAM OF SOME TROPANES⁴

Gas chromatographic conditions as described in Table 8.3 for the 2 % XE-60 column

- 1 = Tropine/pseudotropine
- 2 = 3-Tigloyloxytropine
- 3 = 3,6-Diisovaleryloxytropine/
3,6-Di-2-methylbutyryloxytropine
- 4 = 3,6-Ditigloyloxytropine/
3,6-Diseneciolyoxytropine
- 5 = 3,6-Ditigloyloxy-7-hydroxy-
tropine

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TABLE 8.4

ANALYSIS OF STRAMONIUM, BELLADONNA AND BELLADONNA TINCTURE B.P.⁴

	Alkaloid (%)					
	Stramonium		Belladonna		Belladonna Tincture	
	Partition	GLC	Partition	GLC	Partition	GLC
Total alkaloids	0.258	0.260	0.208	0.203	0.029	0.028
Scopolamine	0.082	0.078	0.000	0.001	0.001	0.000
Hyoscyamine	0.176	0.182	0.208	0.202	0.028	0.028
Hyoscyamine/ Scopolamine	0.465	0.428	-	0.005	-	-
Total alkaloids (British Pharmacopoeia)	not less than 0.25		not less than 0.30		0.028-0.032	

In a number of investigations the methyl phenyl silicone OV-17 has successfully been used for separation and also for quantitative determinations of tropane alkaloids in pharmaceutical preparations^{9,10,11,12,13,14,15}.

Wilms et al.¹⁶ investigated the changes in the content of atropine and scopolamine during the development of *Atropa belladonna* from April to September using a 3 % OV-17 column. Apo-atropine was also found by analysis in amounts varying from 2.3 % to 4.4 % of the total content of atropine, depending on the stage of the development of the plant. Nothing was, however, mentioned about a possible dehydration of atropine to apoatropine during the gas chromatography. Codeine was utilized as an internal standard since homatropine, which has commonly been used by determinations of atropine and scopolamine, was eluted with about the same retention time as apoatropine under the chromatographic conditions employed.

Other stationary phases have also proved to be useful for gas chromatography of tropane alkaloids. For simultaneous quantitative determination of atropine and scopolamine, Solomon et al.¹⁷ applied an SE-30 column (2.5 %). During studies on the metabolism of tropane alkaloids, whereby quantitative determinations of minor alkaloids were carried out, Achari and Newcombe⁴ used columns with SE-30 (2 %), XE-60 (2 %), Versamid (5 %), QF-1 (2.5 %) and PEGS (2.5 %). The best results were obtained with the XE-60 (2 %) column. In 1964 this stationary phase had been successfully employed by Frauendorf and Vogel¹⁸ in a concentration of 5 % for investigations of the alkaloids in extracts of the *Duboisia* species.

As already observed by Brochmann-Hanssen and Baerheim Svendsen², dehydration of atropine and scopolamine may take place under certain experimental conditions. In their gas chromatographic studies on atropine and scopolamine, Solomon et al.¹⁷ made the same observations. They also found that scopolamine hydrobromide was decomposed in some other way, giving several peaks on the chromatogram. Zimmerer Jr. and Grady⁹ worked out an assay of hyoscyamine (atropine) and scopolamine in pharmaceutical preparations and they found that when using properly cured columns, no derivatization of the alkaloids was necessary; standards assayed with precisions of 4.8 % for scopolamine, 6 µg/unit dose, and 2.5 % for hyoscyamine (atropine) at 100 µg/dose. Homatropine served as an internal standard. In another paper, Grady and Zimmerer Jr.¹⁰ stressed the importance of the quality of the column. Improperly or partially cured and conditioned columns often cause extensive tailing of the alkaloids mentioned, and an additional problem can be partial on-column dehydration. A collaborative study of the assay for atropine sulphate tablets, injection and ophthalmic solution, and for scopolamine hydrobromide tablets and injection (USP XVIII) in nine different laboratories, where hom-

atropine was added as internal standard, showed that the method was accurate, reliable, sensitive, highly specific, rapid and reasonable precise.

Santoro et al.¹¹ worked out a similar method for the quantitative determination of scopolamine in pharmaceutical preparations containing *i.a.* phenylpropanolamine and chlorpheniramine after extraction of the free base and using homatropine as an internal standard. The scopolamine content was less than 9 % of the total belladonna alkaloids present in the preparation.

To prevent dehydration and/or decomposition of tropane alkaloids during gas chromatography, Windheuser et al.¹⁹ prepared the trimethylsilyl derivatives of the alkaloids by means of N,O-bis(trimethylsilyl)acetamide in an N,N-dimethylformamide solution of the alkaloids. In this way they carried out quantitative determinations of scopolamine and its acidic and basic degradation products (scopoline, atropic acid, tropic acid and aposcopolamine).

Nieminen²⁰ applied gas chromatography to determine atropine and scopolamine in a number of pharmaceutical preparations: multicomponent tablets, suppositories, injections, ophthalmic solutions and asthma cigarettes containing Stramonium leaves. The separation of the alkaloids from multicomponent preparations was achieved with a Celite column followed by liquid-liquid extraction. Mestranol was used as an internal standard by the gas chromatography. The author did not observe any dehydration or other decomposition of the alkaloids during the gas chromatographic analysis caused by glass wool placed on the top of the column, as stated by other workers. Results of analyses carried out are given in Table 8.5.

TABLE 8.5

ANALYSIS OF ATROPINE AND SCOPOLAMINE IN PHARMACEUTICAL PREPARATIONS²⁰

Dosage form	Declared composition	Amount stated on the label in mg	Found mg
Tablet	Atropine sulphate	0.400	0.397
	Scopolamine hydrobromide	0.210	0.218
	Phenobarbitone	40.0	
Tablet	Total alkaloids of Belladonna leaves	0.200	0.205
	Ergotamine tartrate	0.6	
	Phenobarbitone	40.0	
Tablet	Belladonna extract	10.0	
	corresponding to atropine	0.140	0.142
	Opium extract	5.0	
	Albumin tannate	0.200	
Tablet	Bismuth subsalicylate	400.0	
	Belladonna extract	2.4	
	corresponding to atropine	0.0336	0.0334
	Aloe extract	40.0	
	Sapo medicatus	10.0	
	Resina scammoniae	20.0	
	Rheum extract	20.0	
Injection	Frangula extract	16.0	
	Atropine sulphate	0.250	0.255
	Morphine hydrochloride	20.0	
	Glycerol	50.0	
	Alcohol	120.0	
	Sodium pyrosulphite	1.0	
	Purified water	ad 1 ml	

TABLE 8.5 (continued)

Dosage form	Declared composition	Amount stated on the label in mg	Found mg
Injection	Scopolamine hydrobromide	0.600	0.602
	Morphine hydrochloride	20.0	
	Glycerol	50.0	
	Alcohol	120.0	
	Sodium pyrosulphite	1.0	
	Purified water	ad 1 ml	
Ophthalmic solution	Atropine sulphate	5.0	4.95
	Preservatives		
	Purified water	ad 1 ml	
Ophthalmic solution	Scopolamine hydrobromide	2.5	2.45
	Preservatives		
	Purified water	ad 1 ml	
Suppository	Total alkaloids of Belladonna leaves	0.250	0.247
	Ergotamine tartrate	2.0	
	Caffeine	100.0	
	Allylbarbituric acid	100.0	
	Adeps solidus. Excip.	q.s.	

For the quantitative determination of homatropine methylbromide in tablets and elixirs, Grabowski et al.²¹ hydrolyzed the compound, extracted the free mandelic acid and determined it quantitatively as trimethylsilyl derivative. A glass column packed with 15 % QF-1 on Gas Chrom Q, acid washed and silanized, was used at a column temperature of 160°C. Liebisch et al.²² gas chromatographed tropane alkaloids and related compounds (tropine, pseudotropine, nortropine, scopoline, ecgonine, pseudoecgonine, atropine, cochlearine, scopolamine, meteloidine and benzoylecgonine) as their trimethylsilyl derivatives, using N-methyl-N-trimethylsilyl-trifluoroacetamide as silylation reagent. They utilized a 3 % QF-1 column with Gas Chrom Q at 80°C for the tropanealkamines, 135°C for ecgonine and 185°C for the ester alkaloids.

For analysis of the alkaloids present in *Duboisia* species, Griffin et al.²³ converted the alkaloids after extraction and purification into their trimethylsilyl derivatives to prevent dehydration to the apo-form. Quantitative determinations were carried out on a 1.5 % SE-30 column on acid washed, silanized Chromosorb W, which was precoated with traces of Carbowax 4000 prior to SE-30, as proposed by Brochmann-Hanssen and Baerheim Svendsen².

TABLE 8.6

ANALYSIS OF SAMPLES OF *DUBOISIA MYROPOROIDES* LEAVES²³

Sample	Percentage scopolamine confidence limits p = 0.95			Percentage hyoscyamine confidence limits p = 0.95		
	Amount found	Upper	Lower	Amount found	Upper	Lower
E5/5	0.209	0.223	0.194	0.244	0.257	0.231
E5/15	0.487	0.507	0.468	0.213	0.225	0.201
E5/10	0.415	0.432	0.398	0.163	0.175	0.151
E5/13	0.552	0.575	0.531	0.121	0.134	0.109
F9/5	0.267	0.280	0.254	0.239	0.251	0.227
F9/15	0.351	0.367	0.333	0.169	0.180	0.157
F9/12	0.223	0.236	0.210	0.103	0.115	0.090
G2/6	0.283	0.300	0.265	0.093	0.110	0.075
G2/16	0.414	0.431	0.398	0.078	0.091	0.065
G2/8	0.218	0.231	0.205	0.067	0.080	0.054
G2/11	0.372	0.387	0.357	0.104	0.116	0.092
J11/5	0.573	0.596	0.551	0.073	0.084	0.066
J12/3	0.586	0.611	0.564	0.088	0.100	0.075
J12/10	0.569	0.592	0.547	0.086	0.099	0.073
J12/14	0.587	0.611	0.565	0.220	0.208	0.232

The usefulness of gas chromatography in toxicological analysis - especially in the field of alkaloids - was emphasized by Parker et al.⁵, who also included atropine and scopolamine in their studies. Almost at the same time Kazyak and Knoblock²⁴ carried out an investigation on gas chromatography of a number of drugs in toxicological analysis. Atropine and scopolamine were separated on an SE-30 (1 %) column at temperatures above 200°C. Kolb and Patt²⁵ gas chromatographed atropine on SE-52 (2.5 %) at 200°C. A polar stationary phase (HI-EFF-8B = cyclohexanedimethanol succinate) was applied by Jain and Kirk²⁶ for gas chromatography of several alkaloids including atropine. They also describe an extraction procedure for alkaloids from 0.5 ml blood samples with acetone-diethyl ether without any pH adjustment.

A rapid gas chromatographic method for the analysis of mixtures of tropine and pseudotropine with an absolute accuracy of less than 0.5 % was used by Van der Vlies and Caron²⁷, using 15 % Apiezon L on Chromosorb P pretreated with potassium hydroxide.

A special application in the field of gas chromatography of tropane alkaloids is reaction gas chromatography, whereby the tropane structure in the alkaloids can be determined²⁸. By dehydration on a platinum-firebrick catalyst of tropanol and alkaloids containing a tropanol moiety, the same main products are formed, viz., among others, pyrrole, methylpyrrole, pyrrolidine, pyridine, piperidine and toluene. The gas chromatography was carried out by the authors on a 20 % Carbowax column on base-washed firebrick at 140°C.

Bayne et al.²⁹ developed a gas chromatographic-mass spectrometric method for the determination of scopolamine in plasma and urine samples - sensitive to 50 pg/ml for a 4 ml sample. A deuterated internal standard (scopolamine ($N-CD_3$) hydrobromide hydrate) was used to minimize variability in absolute recovery in the extracting procedure. Scopolamine and deuterated scopolamine were formed from the base-catalyzed hydrolysis of scopolamine and the internal standard, and were analyzed as the heptafluorobutyrate, using a gas chromatographic-mass spectrometric system by monitoring the m/e 138 and 141 fragments, respectively. The heptafluorobutyryl derivative of scopolamine was not used for its sensitivity towards electron-capture detection, but because it chromatographed well on the picogram level and showed a favorable fragmentation pattern under electron-impact ionization. The use of deuterated internal standard for selective ion monitoring negates the necessity for finding an internal standard with similar partition properties, but with different retention times, which is required if electron-capture detection is used.

Further, the use of selective ion monitoring in the gas chromatographic-mass spectrometric method greatly reduces the problem of biological interference as compared to electron-capture detection. The gas chromatography of the heptafluorobutyryl derivatives of scopolamine was carried out either on a 6 feet by 2 mm I.D. glass column packed with 3 % OV-17 or 1 % OV-225 on Gas Chrom Q (200-120 mesh) at 95°C.

Wyatt et al.³⁰ worked out a GLC assay for atropine and scopolamine in belladonna extract. The extract was solved in 0.1 N sulphuric acid, homatropine hydrobromide was added to this solution as an internal standard, and interfering materials were extracted from the acidified solution with chloroform - and finally a mixture of chloroform and 2-propanol (10:3) if there is an emulsion problem. The alkaloids were subsequently extracted into chloroform (or chloroform-2-propanol) from the basified aqueous layer (pH 9.5 phosphate buffer was used instead of mineral alkali to minimize ester cleavage) and the chloroform extracts were filtered through anhydrous sodium sulphate (previously washed with chloroform). 87 % of the alkaloids were recovered in the first extract, so that two additional extractions gave suffi-

cient alkaloid recovery. Pure sodium sulphate should be used since small amounts of impurities, such as calcium, were found to catalyze on-column decomposition of the atropine sample with gas chromatography. Two OV-17 columns were prepared: on the first, preferential decomposition to apoatropine was observed; on the second the atropine peak was severely distorted only. Assay values are given in Table 8.7.

TABLE 8.7

CONTENT OF ATROPINE, SCOPOLAMINE AND TOTAL ALKALOIDS IN BELLADONNA EXTRACTS DETERMINED BY GLC³⁰

Declared content: 12.5 mg/g of total alkaloids

Sample		Weighing	Atropine mg/g	Scopolamine mg/g		Total alkaloids mg/g		
Pilular extract 1		A	12.69	12.59	0.29	0.31	12.81 \pm 0.14 (RSD = 1.08 %)	
		B	12.59	12.65	0.29	0.30		
		C	12.57	12.64	0.30	0.30		
		D	12.48	12.44	0.29	0.28		
		E	12.25	12.34	0.29	0.29		
		(average = 12.51 \pm 0.13 RSD = 1.07 %)		(average = 0.29 \pm 0.01 RSD = 2.87 %)				
		2	A	11.21	11.20	0.50	0.50	11.71 \pm 0.06 (RSD = 0.54 %)
			B	11.18	11.20	0.49	0.50	
			C	11.12	11.18	0.48	0.49	
			D	11.30	11.22	0.50	0.49	
E	11.30		11.17	0.52	0.53			
	(average = 11.21 \pm 0.06 RSD = 0.49 %)		(average = 0.50 \pm 0.01 RSD = 2.59 %)					
	Powdered extract 1		A	11.48	11.34	0.20	0.20	11.56 \pm 0.07 (RSD = 0.63 %)
			B	11.36	11.34	0.18	0.22	
			C	11.36	11.36	0.18	0.22	
			D	11.26	11.47	0.20	0.21	
E			11.32	11.28	0.22	0.19		
		(average = 11.36 \pm 0.07 RSD = 0.62 %)		(average = 0.20 \pm 0.02 RSD = 7.71 %)				
		2	A	11.64	11.58	0.11	0.11	11.79 \pm 0.12 (RSD = 1.02 %)
			B	11.58	11.58	0.09	0.11	
			C	11.74	11.59	0.09	0.09	
			D	11.76	11.68	0.10	0.11	
E	11.91		11.80	0.12	0.12			
	(average = 11.69 \pm 0.11 RSD = 0.97 %)		(average = 0.11 \pm 0.01 RSD = 11.80 %)					

Göber et al.³² applied gas chromatography for an investigation of the stability of scopolamine hydrobromide in eye-drops. During heat-sterilisation of such solutions, scopolamine and tropic acid, as well as aposcopolamine, may be formed. By converting these compounds into their trimethylsilyl derivatives with N,O-bis(trimethylsilyl)acetamide as silylating reagent, good results could be obtained, as can be seen in the chromatogram in Figure 8.2. Atropine was used as an internal standard and the gas chromatography was performed on a packed 2 m long column with 10 % SE-30 on silanized Chromosorb W, with temperature programming.

The U.S.P. XX³³ introduced gas chromatography for the assay of atropine in various atro-

pine sulphate containing preparations (Injection, Ophthalmic solution, Ophthalmic ointment and Tablets) using a 1.8 m long by 2 mm I.D. packed column with 3 % of a mixture of phenyl- and methylpolysiloxane (1:1) as stationary phase on "siliceous earth for gas chromatography". Homatropine was used as an internal standard for the assay, which was carried out at a column temperature of 225°C. Gas chromatography was also applied for the assay of Belladonna leaf, extract and tincture with homatropine as an internal standard. However, in this case a shorter column (1.2 m) and a column temperature of 215°C were used.

Also the British Pharmacopoeia 1980³⁴ describes an assay for atropine in atropine sulphate eye drops and tablets, as well as in Hyoscyamus dry extract. However, atropine was silylated with N,O-bis(trimethylsilyl)acetamide and trimethyldichlorosilane (4:1) and gas chromatographed on a 1.5 m long by 4 mm I.D. packed with 3 % OV-17 on "diatomaceous support" AWS at 200°C using homatropine as an internal standard.

Majláč³⁵ worked out a gas chromatographic assay of atropine and phenobarbital in pharmaceutical preparations containing Valeriana liquid extract. After extraction of atropine, it was hydrolyzed and the free tropic acid silylated with N,O-bis(trimethylsilyl)acetamide. The assay was performed on a glass column, 1 m long by 3 mm I.D. packed with 1.5 % OV-101 on silanized Gas Chrom P, 100-120 mesh, at 140°C, using silylated 2-naphtol as an internal standard.

FIGURE 8.2

GAS CHROMATOGRAM OF TROPINE ALKALOIDS AND DERIVATIVES AS TRIMETHYLSILYL DERIVATIVES³²

Scopoline (1), tropic acid (2), aposcopolamine (3), atropine (4) and scopolamine (5); column: 2 m long, 3 mm I.D.; 10 % SE-30 on Chromosorb W; temperature programming 100-240°C

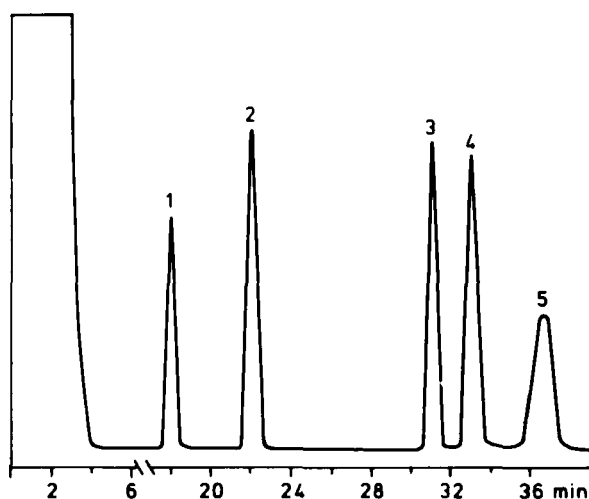


TABLE 8.8

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF TROPINE ALKALOIDS

Column	Solid support mesh	Stat.phase	%	Temperature	Comp.Prep.	Ref.
glass, 6 ft x 4 mm	CW 80-100	SE-30	2-3	204°C	alk. s.	1
glass, 6 ft x 3 mm	GP ABS + PEG 100-140	SE-30	1.15	175°C	alk. pm.	2
s.s., 5 ft x 1/8 in	Aer 100-120	SE-30	5	175°C	alk. pm.	3
glass, 1.52 m x 5 mm I.D.	CWS 80-100	XE-60	10	200°C		
s.s., 1.52 x 5 mm I.D.					alk. pm.	4
glass, 1.52 m x 5 mm I.D.	CWS 80-100	QF-1 + XE-60	2.5 + 2.5	200°		
s.s., 1.52 m x 5 mm I.D.						
5 ft x 0.093 in I.D.	CWS AW 80-80	SE-30	5	210°C	tox.	5
glass, 3 ft x 2 mm	CP AWS 80-100	NGS	1	230°C		
glass, 3 ft x 2 mm	CP AWS 80-100	NGS + PVP	1 + 1	230°C	alk. s.	6
glass, 3 ft x 2 mm	CP AWS 80-100	SE-30	1	195°C and 220°C		
-	-	XE-60	1	220°C	alk. s.	7
-	-	EGSSY	1	230°C		
-	-	HI-EFF 8B	1	230°C and 240°C		
2 m	CG AWS 80-100	SE-30	2		alk. tox.	8
1 m	CG AWS 80-100	OV-17	5			
glass, 60 cm x 3 mm I.D.	GQ	OV-17	3	210°C	alk.qnt.prep.	9
glass, 60 cm x 4 mm I.D.	GQ AWS 80-100	OV-17	3	210°C	alk. prep.	10
glass, 1.2 m x 4 mm I.D.	GQ AWS 80-100	OV-17	3	225°C		
glass, 1.2 m x 4 mm I.D.	GQ AWS	OV-17	3	210°C	alk. prep.	11
2.8 m x 4 mm I.D.	GQ 100-120	OV-17	3	240°C	alk.qnt.pm/	12
glass, 1.45 m x 3.8 mm	GQ 80-100	OV-17	3		alk.pm.	13
glass, 1.45 m x 3.8 mm	GQ 80-100	SE-30	30			
glass, 1.45 m x 3.8 mm	GQ 80-100	OV-17	3	150-230°C pr. 6°C/min	alk.pm.	15
glass, 3 ft x 3 mm	GQ 100-120	OV-17	3	90-250°C pr.	alk.pm.	16
6 ft x 0.19 in I.D.	CG AW 80-100	SE-30	2.5	150-275°C pr. 6°C/min	alk.qnt.prep.	17
s.s., 60 cm x 4.5 mm I.D.	CWS 80-100	XE-60	5	110-210°C pr.	alk. s.	18
glass, 2.4 m x 4 mm	GQ AWS 80-100	OV-17	1	100-220°C pr.	scop.decomp.pr.	19
glass, 1.2 m x 4 mm I.D.	Diat S 80-100	SE-30	3.5	215°C	TMS deriv.	20
glass, 1.52 m x 3.2 mm I.D.	GQ AWS	QF-1	15	160°C	alk.prep.	21
glass, 1.5 m x 4 mm I.D.	GQ	QF-1	3	85-185° pr	TMS	
glass, 1.5 m x 4 mm I.D.	CW AWS + PEG 80-100	SE-30	1.5	194°C	alk.TMS deriv.	22
glass, 6ft x 4 mm I.D.	Ana ABS 100-120	QF-1-0065	3	200°C	alk.pm.	23
		SE-52	2.5			
		HI-EFF-8B				
glass, 1.2 m x 4 mm I.D.	CP BW 175-210 μ	Apiezon L	15	132°C	alk. tox.	24
glass, 2.25 m x 6 mm I.D.	Fir. BW	Cab 20M	20	140°C	pseudotropine/ tropine	25
glass S, 1.8 m x 2 mm I.D.	GQ 100-120	OV-17	3	95°C	reaction GLC	26
-	-	OV-225	1		scop.pl.ur.as	27
					scopl.deriv.	28
					GC-MS	29
glass, 1.2 m x 4 mm I.D.	GQ AW 100-120	OV-17	3	215°C	alk.pm.extr.	30
s.s., 2 m x 3 mm I.D.	CW AWS 80-100	SE-30	10	100-240°C pr.	scop. decomp. pr. TMS	31
glass, 1.8 m x 2 mm I.D.	SiEG	P+M sil(1:1)	3	225°C	atr. qnt.prep.	32
glass, 1.2 m x 2 mm I.D.	SiEG	P+M sil(1:1)	3	215°C	alk. pm.	33
glass, 1.5 m x 4 mm I.D.	Diasup. AWS	OV-17	3	200°C	atr. qnt.prep.	34
glass, 1 m x 3 mm I.D.	GP S 100-120	OV-101	1.5	140°C	atr. as trop.a. sil.der.	35

Abbreviations:

ABS = acid, base washed, silanized
 AW = acid washed
 Aer = Aeropak
 alk = alkaloid
 Ana = Anakrom
 atr = atropine
 BW = base washed
 Cab = Carbowax
 CW = Chromosorb W
 decomp. pr. = decomposition product
 der = derivative
 Dia S = Diatoport S
 Diasup = diatomaceous support
 extr = extract
 Fir = firebrick
 GP = Gas Chrom P
 GQ = Gas Chrom Q
 I.D. = inside diameter

M silx = methylpolysiloxane
 pl = plasma
 pm = plant material
 P silx = phenylpolysiloxane
 prep = pharmaceutical preparation
 pr = (temperature) programming
 qnt = quantitative
 S = silanized
 s = separation
 sco = scopolamine
 scopl = scopolone
 SiEG = siliceous earth for GC
 sil = silylated
 s.s. = stainless steel
 tox = toxicology
 TMS = trimethylsilyl
 trop.a = tropic acid
 ur = urine

TABLE 8.9

SILYLATION OF SOME TROPINE ALKALOIDS AND RELATED COMPOUNDS

1. With N,O-bis(trimethylsilyl)acetamide

To the sample (1.5 mg scopolamine, 0.5 mg scopolone, atropic acid) add 400 μ l of N,N-dimethylformamide and 400 μ l N,O-bis(trimethylsilyl)acetamide, stopper tightly, and swirl until complete dissolution¹⁹.

Add 200 μ l of the silylating agent to the dry sample (0.6 mg homatropine methylbromide or 0.2 mg mandelic acid) in a cone-shaped vial, cover and allow to stand at room temperature for 20 minutes with occasional shaking²¹.

2. With N-methyl-N-trimethylsilyl-trifluoroacetamide

Add a small excess of N-methyl-N-trimethylsilyl-trifluoroacetamide to the dry free base or to the base in benzene solution and heat to 80°C for a short time²².

3. With hexamethyldisilazane

Solve the dry free base in pyridine containing 5 % hexamethyldisilazane and allow to stand until next day²³.

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Chapter 9

PSEUDOTROPINE ALKALOIDS

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9.1. COCA ALKALOIDS

The gas chromatography of cocaine has mainly been dealt with in connection with investigations to develop selective and sensitive methods for its detection and quantitative determination in pharmacological and toxicological analysis. One study has so far been done on naturally occurring alkaloidal mixtures present in the coca plant (*Erythroxylon coca* Lam.)¹.

The gas chromatography of cocaine has mostly been carried out on packed columns with non-polar stationary phases. Silicone rubber SE-30 was utilized by Lloyd et al.², Brochmann-Hanssen and Baerheim Svendsen³, Parker et al.⁴, Brochmann-Hanssen and Fontan⁵, as well as by Koontz et al.⁶, whereas SE-52 was used by Kolb and Patt⁷, OV-1 by Blake et al.⁸ and OV-101 and OV-25 by Moore⁹. However, stationary phases of various polarities have also been used successfully, such as silicone nitrile gum (XE-60), polyester methylsilicone copolymer (EGSS-Y), and cyclohexanedimethanol succinate polyester (HI-EFF-8B) by Brochmann-Hanssen and Fontan⁵, and Jain and Kirk¹⁰; neopentylglycol sebacate (NGSE) by Kolb and Patt⁷, as well as neopentyl glycol succinate (NGS) by Brochmann-Hanssen and Fontan¹¹.

1-2 m long packed columns with 1-2 % stationary phase and column temperatures of about 175-240°C were used to obtain reasonable retention times for cocaine.

For a gas chromatographic identification of alkaloids, Brochmann-Hanssen and Fontan⁵ recommended a combination of two columns, one non-polar (SE-30) and one semi-polar (XE-60). Hammer et al.¹² recommended, for a definitive GLC identification of cocaine in laboratories where GC-MS apparatus is available, an on column methylation of cocaine by means of trimethylanilinium hydroxide, whereby cocaine is converted into ecgonidine methyl ester, which appears as the major peak on the gas chromatogram on an OV-17 column, 2 feet long at 250°C, in addition to N,N-dimethylaniline, a product derived from trimethylanilinium hydroxide during the methylation reaction.

Scaringelli¹³ separated cocaine and a number of other "caines" (Procaine, Tetracaine, Benzocaine) on a 2 feet long silicone rubber column (10 %) at 220-225°C, and with temperature programming, starting at 75°C. He collected the separated compounds and identified them by their special crystal tests. Estimation of cocaine was obtained by using benzocaine as an internal standard.

For a quick detection of cocaine in mixtures with other compounds, such as amphetamines, other "caines" etc., Cavallaro et al.¹⁴ applied gas chromatography on three columns of different polarity (SE-30, Triton X 305 and QF-1). In this way the identity of the various compounds could be established via their retention times.

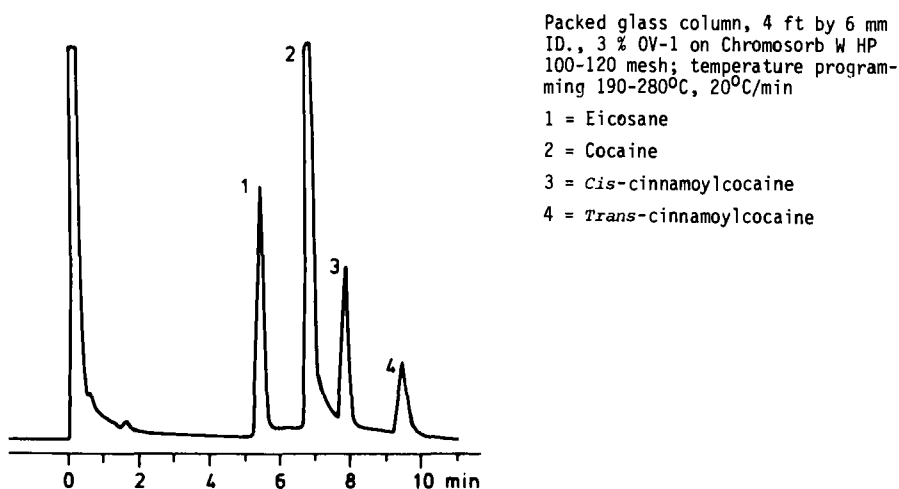
Detection and determination of small amounts of minor alkaloids and/or degradation products of cocaine in illicit cocaine samples may be of importance in tracing domestic and international clandestine cocaine routes. Moore¹⁵ developed a gas chromatographic method for the determination of *cis*- and *trans*-cinnamoylcocaine in illicit cocaine samples, in which the

cinnamylcocaine may be present in concentrations of 1 % or less of the amount of cocaine. The gas chromatographic analysis was carried out after silyl treatment on packed columns with 3 % OV-1 on Chromosorb W HP with temperature programming, using eicosane as an internal standard. A typical chromatogram is given in Figure 9.1.

FIGURE 9.1

GAS CHROMATOGRAM OF ILLICIT PERUVIAN COCA PASTE AFTER SILYL TREATMENT¹⁵

Eicosane used as an internal standard.



An official gas chromatographic method for quantitative determination of cocaine hydrochloride was described in J. Assoc. Off. Anal. Chem., 61 (1978) 473¹⁶, whereby cocaine is extracted from a weakly basic aqueous solution, with chloroform containing the internal standard, tetracosane. A 6 feet by 4 mm inside diameter glass column packed with 3 % OV-1 on 100-120 mesh Chromosorb W HP was utilized at column temperature 225°C.

In pharmaceutical cocaine the hydrolysis products, benzoylecgonine and ecgonine may be present as impurities. In toxicological studies these two compounds are found as the main metabolites of cocaine. Several workers have gas chromatographed benzoylecgonine and ecgonine after derivatization, due to the very poor gas chromatographic behaviour of the two compounds. Moore⁹ proposed silylation by means of N,O-bis(trimethylsilyl)acetamide prior to GLC on 10 % OV-101 and 3 % OV-25 columns, using temperature programming. When using the OV-25 column, the elution order of cocaine and the benzoylecgonine silyl derivative was reversed, compared to OV-101. The OV-25, therefore, was used as a confirmation of the presence of ecgonine and benzoylecgonine in cocaine. OV-101 was the column of one's first choice because the resolution, sensitivity and the retention times of cocaine and its hydrolysis products were the most favorable, as will be seen in Table 9.1.

Moore was able to detect ecgonine and benzoylecgonine in cocaine samples at levels less than 0.1 % and 0.3 %, respectively, as silyl derivatives.

TABLE 9.1

RETENTION TIMES OF COCAINE, THE SILYL DERIVATIVES OF ECGONINE, BENZOYLECGONINE AND INTERNAL STANDARDS⁹

Columns: 4 ft x 4 mm I.D., 10 % OV-101 on Chromosorb W HP; 6 ft x 4 mm I.D., 3 % OV-25 on Chromosorb Q.

Compound	Retention time (min)	
	OV-101	OV-25
Hexadecane - internal standard	3.8	-
Ecgonine silyl derivative	6.7	2.8
Eicosane - internal standard	-	5.2
Cocaine	19.7	25.8
Benzoylecgonine silyl derivative	22.2	24.5
Tetracosane - internal standard	26.7	-
Triacotane - internal standard	-	30.2

Fish and Wilson¹⁷ worked out a method for the determination of cocaine in urine by extraction of the urine with diethyl ether after addition of hydrochloric acid to remove impurities, followed by addition of sodium bicarbonate to give pH 8 (approx.) and further extraction of the cocaine with diethyl ether, concentration of the diethyl ether extract and GLC. Benzhexol was utilized as an internal standard. The reproducibility of the gas chromatographic determination of cocaine extracted from urine is seen in Table 9.2.

TABLE 9.2

REPRODUCIBILITY OF THE GAS CHROMATOGRAPHIC DETERMINATION OF COCAINE EXTRACTED FROM URINE¹⁷

	Concentration (ug/ml)		Number of determinations
	Actual	Found	
Cocaine	3.25	3.01 ± 0.09	10
	13.0	12.91 ± 0.39	10

The same authors¹⁸ studied the excretion of cocaine and its metabolites in man; cocaine was extracted from urine with diethyl ether and the benzoylecgonine with chloroform, to which the internal standard (5 α -cholestane) was added. Benzoylecgonine was converted into its methyl ester (cocaine) by means of diazomethane, and the gas chromatography was carried out as described by the same authors in the paper referred to above¹⁷.

Wallace et al.¹⁹ determined benzoylecgonine in urine after extraction and methylation to cocaine. Separate simultaneous determinations of cocaine and benzoylecgonine were achieved by analyzing both a methylated (combined cocaine and benzoylecgonine) and a non-methylated (cocaine only) aliquot of the specimen extract on a 1 m by 3 mm I.D. OV-17 column on Supelcoport 80-100 mesh at 220°C. The recovery from biological specimens of 93 and 65 % for cocaine and benzoylecgonine, respectively, and 73 % conversion of benzoylecgonine to cocaine, provided detection limits of 0.1 and 0.2 $\mu\text{g/ml}$ for cocaine and benzoylecgonine, respectively.

For the determinations of benzoylecgonine in urine Koontz et al.⁶ salted out the compound of the urine with K_2HPO_4 and KH_2PO_4 into 95 % ethanol, evaporated the ethanol extract to dryness and purified the benzoylecgonine by thin-layer chromatography. The compound was then removed from the thin-layer plate, methylated with dimethylformamide dimethyl acetal and determined by gas chromatography.

TABLE 9.3

RECOVERY OF COCAINE AND BENZOYLECGONINE FROM URINE¹⁹

Cocaine		Benzoylecgonine	
Added to urine μg/ml	Amount determined μg/ml *)	Added to urine μg/ml	Amount determined μg/ml *)
0.25	0.20 ± 0.05	0.5	0.32 ± 0.05
0.5	0.50 ± 0.05	1	0.69 ± 0.05
1	1.06 ± 0.09	2.5	1.56 ± 0.08
2.5	2.18 ± 0.24	5	3.14 ± 0.40
5	4.63 ± 0.14	10	6.29 ± 1.23
		20	13.56 ± 0.54

*) Mean of quadruplet determination ± standard deviation

To achieve a rapid, sensitive and relatively specific gas chromatographic screening procedure for cocaine, Blake et al.⁸ reduced cocaine with LiAlH_4 to 2-hydroxymethyl tropine, O-acylated this compound by treating it with pentafluoropropionic anhydride or heptafluorobutyric anhydride and chromatographed the derivative formed using an OV-1 3 % on Chromosorb C HP 80-100 mesh column (4 ft by 4 mm I.D.) at 150°C and an electron capture detector which greatly increased the sensitivity of the method. The procedure used by Blake et al. for the determination of cocaine in urine involved a 2.5-fold concentration factor in extraction, and employing the pentafluoropropionic anhydride derivative, a sample containing 39 ng/ml cocaine in urine gave an average S/N ratio of 14:1 for 1.0 μl injected. Therefore, sensitivities of 20-30 ng of drug/ml of the sample could be achieved without concentration through evaporation procedures.

Javaid et al.²⁰ applied practically the same method to determine quantitatively cocaine and its metabolites benzoylecgonine and ecgonine. The method involved the formation of acyl derivatives which were separated on 3 % and 5 % OV-1 columns and detected in picomole quantities using an electron capture detector. Ecgonine and benzoylecgonine were derivatized with a mixture of hexafluoroisopropanol and heptafluorobutyric anhydride (1:2). Cocaine was first reduced by LiAlH_4 and then acylated by pentafluoropropionic anhydride. Benzoylecgonine, but not ecgonine, could also be determined by reduction and subsequent acylation. This provided the basis for the determination of cocaine, benzoylecgonine and ecgonine from the same sample. Cocaine could be determined in urine and plasma by this method. The results obtained with the method are given in Table 9.4

TABLE 9.4

SIMULTANEOUS DETERMINATION OF COCAINE, ECGONINE AND BENZOYLECGONINE IN THE SAME SAMPLE²⁰

	Concentration (μg/ml)	
	Calculated	Determined *)
Cocaine	0.25	0.21
Ecgonine	0.1	0.12
Benzoylecgonine	3.0	2.9

*) These values are the average of three determinations

Javaid et al.²¹ extended their method described above²⁰ for the determination of cocaine, to its determination in urine, plasma and red blood cells of volunteer subjects who were

given different doses of cocaine intravenously. At slightly alkaline pH, cocaine was extracted with cyclohexane, reduced to 2-hydroxymethyltropine with LiAlH_4 , acylated with pentafluoropropionic anhydride and gas chromatographed using an electron capture detector. The recoveries from urine (95-102 %), plasma (65-80 %) and red blood cells (60-70 %) were the same at the pH range of 6.5 to 9.5. Above pH 10 recoveries were greatly reduced, due to chemical hydrolysis of cocaine to benzoylecgonine.

Berry and Grove²² applied gas chromatography for cocaine base as a confirmatory test for its detection in urine samples after a simple pH adjustment and extraction with organic solvent. As a confirmation test in general - especially for TLC results for hospital drug emergencies as well as for qualitative and quantitative analysis of narcotic drugs from biological material - gas chromatography is an ideal method²³.

For the determination of nine CNS drugs, *i.e.* cocaine, codeine, methadone, in human plasma, Medzihradsky and Dahlstrom²⁴ used gas chromatography. The drugs were extracted with benzene-isopropanol (9:1) from 1 ml plasma after adjustment of the pH to 10. The benzene-isopropanol extract was evaporated to dryness and the residue dissolved in 25-50 μl acetone. 2 μl of the solution was injected into the gas chromatograph. The recovery of the drugs, 0.25-4 $\mu\text{g/ml}$, was 80-100 %, the lower limit of sensitivity 3 ng to 6 ng, corresponding to concentrations in plasma of 0.05 $\mu\text{g/ml}$ and 0.13 $\mu\text{g/ml}$ respectively.

To be able to determine small quantities of cocaine in blood plasma following its topical application to mucous membranes, Dvorchik et al.²⁵ developed a method using a nitrogen sensitive flame ionization detector. Aliquots (1 or 2 ml) of whole blood or plasma were made alkaline with carbonate buffer and extracted with benzene-isopropanol (9:1), the cocaine was extracted back with 0.01 N hydrochloric acid, the aqueous phase adjusted to pH 7-7.3 and the cocaine base extracted with benzene-isopropanol. After evaporation to dryness the residue was dissolved in acetone and the solution obtained used for gas chromatographic analysis: 20 ng of cocaine from 1 ml whole blood or plasma could be determined by this method on a SP-2100-DB 3 % column. The mean analytical recovery was 65 %.

In a comparison of the methods employed for the detection of *i.e.* cocaine and metabolites in toxicological analysis, Bastos and Hoffman²⁶ dealt with the extraction procedure from biological material and the subsequent gas chromatography. They concluded that cocaine may be reduced and acylated before gas chromatographic analysis, but that it does not require derivatization. However, its metabolites, benzoylecgonine and ecgonine, do require esterification with dimethyl acetal, N,N-dicyclohexylcarbodiimide, or with a similar reagent. The resulting compounds are normally separated at temperatures in the range 215-240°C. Chromatographic columns of SE-30 on Chromosorb W AW and silanized have been used for the analysis of cocaine as well as for the alkyl derivatives of benzoylecgonine and ecgonine. The use of OV-1 has become popular for the analysis of basic drugs, thus providing an alternate system to SE-30. Also, OV-17 has been used successfully. The results obtained by gas chromatography should be confirmed by alternative procedures. This could involve performing the GC analysis under different operating conditions, or employing techniques that can be linked to a GC system. Such techniques may range from microcrystal tests to the more sophisticated infrared spectrometry and mass spectrometry.

Because of the limited sensitivity of a conventional flame-ionization detector and its non-selective response to co-extracted endogenous constituents, Jatlow and Bailey²⁷ utilized a nitrogen detector for gas chromatographic assay of cocaine in plasma. They achieved the de-

termination of cocaine in plasma at concentrations as low as 5 µg/liter using benzoylecgonine-*n*-propylester as an internal standard. Despite the selective response of the nitrogen detector, the authors found it desirable to use a back extraction and clean-up step to eliminate *i.e.* phospholipids. The more common "drugs of abuse" and commonly used local anesthetics did not interfere, but serious sources of interference were found to be contaminants on glass ware, rubber stoppers and supposedly pure solvents.

Kogan et al.²⁸ determined cocaine and its principal metabolite in man, benzoylecgonine, by using gas chromatography and (a) electron capture detection for the determination of benzoylecgonine from plasma as its pentafluorobenzyl derivative, (b) FID for the determination of benzoylecgonine and cocaine from urine as trimethylsilyl derivatives, and (c) nitrogen detection for the determination of cocaine from plasma. The limits of detection for cocaine, undervatized, and benzoylecgonine as its pentafluorobenzyl derivative in plasma, were 10 and 5 ng/ml, respectively. In urine the sensitivity limits of the silyl derivatives of cocaine and benzoylecgonine were 0.5 and 1.0 µg/ml, respectively. The coefficient of variation ranged between 0.9 and 2.2 % and the coefficient of determination was 0.99 for the method used.

For the determination of benzoylecgonine from plasma, the compound was extracted after basification (pH 9.5) with ethanol-chloroform (20:80), then it was immediately reacted with pentafluorobenzyl bromide and the pentafluorobenzyl-benzoylecgonine partitioned into a non-polar solvent (benzene) leaving the more polar interfering substances behind. This is the important step in the procedure.

Chlorproethazine proved to be a very reliable internal standard; it does not react with the derivatizing reagent, is easily back-extracted into acid, is linear over a 100-fold concentration range and is easily resolved on the gas chromatograph.

A method for simultaneous determination of cocaine and benzoylecgonine in urine was developed by Von Minden and D'Amato²⁹, whereby the compounds were extracted from urine into ethanol-chloroform (25:75) followed by propylation of benzoylecgonine using propyliodide and a mixture of trimethylphenylammoniumhydroxide (0.1 M in methanol) and tetramethylammoniumhydroxide (25 % in methanol) in *N,N*-dimethylacetamide; *n*-pentylbenzoylecgonine was utilized as an internal standard. Since the recovery from biological samples was 99 and 80 % for cocaine and benzoylecgonine, respectively, and 98 % of the benzoylecgonine was converted into its *n*-propylester, the detection limit of 0.2 µg/ml in 5 ml urine was obtained.

A procedure for the simultaneous determination of cocaine and benzoylecgonine in urine specimens was developed by Jain et al.³⁰. The drug was extracted with chloroform-isopropanol from urine samples saturated with a bisalt-buffer. The organic extract was evaporated to dryness, and an aliquot of the residue injected onto the gas chromatograph to determine the presence of cocaine and the location of any extraneous peaks. Depending on the chromatogram obtained, benzoylecgonine was converted by on-column derivatization into an alkyl ester, which was eluted on the chromatogram undisturbed by other compounds present in the extract. The relative retention times of benzoylecgonine esters formed by on-column derivatization from the utilized *N,N*-dimethylformamide dimethyl-, diethyl-, dipropyl-, dibutyl-, di-*tert*-butyl, and dicyclohexyl-acetals are given in Table 9.5

To be able to determine unchanged cocaine and benzoylecgonine excreted in human urine in amounts that are generally below the limits of detection, Jindal and Vestergaard³¹ used gas chromatography-mass spectrometry and stable isotope labeled analogs (cocaine-*d*₃ and benzoyl-

TABLE 9.5

RELATIVE RETENTION TIMES OF BENZOYLECGONINE ESTERS FORMED BY DIMETHYLFORMAMIDE-DIALKYL ACETALS³⁰

Column: 3 ft by 2 mm I.D., 3 % OV-17 on Chromosorb W HP 80-100 mesh at 200°C

Ester	Retention time (sec)	Relative retention time
Methyl (= cocaine)	147	1.00
Ethyl	181	1.27
Isopropyl	192	1.31
<i>n</i> -Propyl	239	1.63
<i>ter</i> -Butyl	246	1.67
<i>n</i> -Butyl	329	2.24
Cyclohexyl	849	5.78

ecgonine- d_3 , both N-methylated- d_3) as internal standards. The assay utilized ion focussing to monitor in the GLC-effluent the molecular ions of cocaine and benzoylecgonine generated by electron-impact ionization. The assay can measure 2 ng of cocaine/ml and 5 ng of benzoylecgonine/ml with about 5 % precision. A magnetic sector, single-focussing mass spectrometer (LKB 9000) interfaced with a gas chromatograph and equipped with a multiple ion detector/peak matcher accessory (MID/PM) was used. The assay of cocaine and benzoylecgonine is sensitive, specific and also applicable to other body fluids and tissues.

In a paper on chromatographic quantitation of cocaine, Roberson³² pointed out some problems concerning quantitative determinations with internal standards. When injecting a chloroform solution of cocaine hydrochloride and an internal standard into the injection port with a syringe, the chloroform is immediately boiled away, leaving a deposit of cocaine hydrochloride and the internal standard on the inside of the needle. Cocaine hydrochloride is not readily soluble in chloroform. The deposit is partially washed away on expulsion of the remainder of the solution by depression of the plunger. The washing process is not reproducible and gives rise to variance in the results. The ideal situation is, therefore, that both analyte and internal standard are very readily soluble in the solvent to be used; eventually an improved reproducibility can be achieved by lowering the amounts of cocaine hydrochloride in the solution while keeping the concentration of the internal standard (readily soluble in chloroform) constant.

Because methylecgonine has been found to be a prominent urinary metabolite of cocaine, *i. a.* after street use of cocaine, which primarily involves intranasal application and intravenous injection rather than ingestion, Ambre *et al.*³³ developed a GC-MS method for the determination of methylecgonine in urine. The identification of this metabolite in the urine is an efficient and reliable means of detecting cocaine use. Samples of 0.5 or 2 ml urine were adjusted to pH 8.5-9 and phencyclidine was added as an internal standard. The sample was then extracted with methylene chloride:isopropanol (3:1), the organic phase separated and taken to dryness. The residue was redissolved in isopropanol or ethanol and analyzed by GC-MS in the selected ion mode. A 74 cm long, 2 mm I.D. packed glass column with 2 % OV-101 on Gas Chrom Q AWS and temperature programming 140-240°C, 15°C/min was used.

For detection of cocaine, analysis of methylecgonine has advantages over that of benzoylecgonine. Methylecgonine can be gas chromatographed directly on common gas chromatographic stationary phases without derivatization, and its early elution shortens analysis time. However, because of interference of other compounds in a urine analysis, the combination of GC

and MS is indispensable. The assay sensitivity of methylecgonine and cocaine by GC-MS using selected monitoring was found to be 0.1 µg/ml when extracting a 2 ml urine sample. Extraction recovery at 5 and 10 µg/ml was 51 % for methylecgonine and 89 % for cocaine.

Graas and Watson³⁴ developed a GC-MS-COM method for the determination of benzoylecgonine in urine following a one-step extraction and derivatization technique, an extractive alkylation technique. A 2 ml sample of urine was made basic with sodium hydroxide containing tetrahexylammonium hydrogen sulphate (THA), and the ion pair benzoylecgonine-THA was extracted into a 5 ml solution of iodoethane in methylene chloride. Using this extraction procedure, 70 % of the benzoylecgonine was converted to the ethyl ester. Residual amounts on THA caused gas chromatographic interferences resulting in broad solvent peaks. Therefore, the THA residue was first dissolved in toluene, then the benzoylecgonine was removed quantitatively and without any major interferences from THA by a liquid-liquid extraction into hexane. The final volumes used were optimized so that no final concentration step was necessary. Linearity was demonstrated from 1 to 50 µg/ml. Quantitation was accomplished by monitoring multiple mass spectral fragments with a precision of 6 % (coefficient of variation) or less.

In a collaborative study on the quantitative determination of cocaine hydrochloride in powders and tablets that also contain other drugs (*i.e.* caffeine, procaine hydrochloride and benzocaine) as well as lactose, mannitol and starch, the free cocaine base was extracted with chloroform from an aqueous solution after addition of K_2HPO_4 . Tetracosane ($n-C_{24}$) was added in the extracting chloroform as an internal standard. Since cocaine base and tetracosane are both readily soluble in chloroform, none of the problems mentioned by Roberson³² were encountered. The recoveries ranged from 98.7 to 103 % for cocaine hydrochloride amounts ranging from 6 to 100 %, with a coefficient of variation from 0.89 to 3.16³⁵.

For the assay of cocaine in *Erythroxylon coca* Lam. from three locations in Peru, Turner et al.¹ worked out a gas chromatographic method. The alkaloids were extracted by refluxing the powdered leaf samples (1.00 g) with ethanol (40 ml) for 15 minutes. The filtrate was evaporated to dryness under reduced pressure in a rotary evaporator, the residue dissolved in 20 ml chloroform and the alkaloids extracted into 1.5 % aqueous citric acid. The pH of the solution was adjusted to 8.2 with sodium bicarbonate and the alkaloid bases extracted with chloroform. After evaporation the residue was dissolved in ethanol containing the internal standard (androst-4-ene-3,17-dione) and gas chromatographed on a packed column of 6 % OV-1 on Chromosorb W. A typical chromatogram is shown in Figure 9.2 and the results of the analysis in Table 9.6.

TABLE 9.6

COCAINE CONTENT IN *ERYTHROXYLON COCA* Lam.¹

Species	Content (%) (*)		Coefficient of variation (%) (**)
Cuzco	0.60	0.03	5.0
Trujillo	0.60	0.03	5.0
Tingo Maria	0.57	0.01	1.8

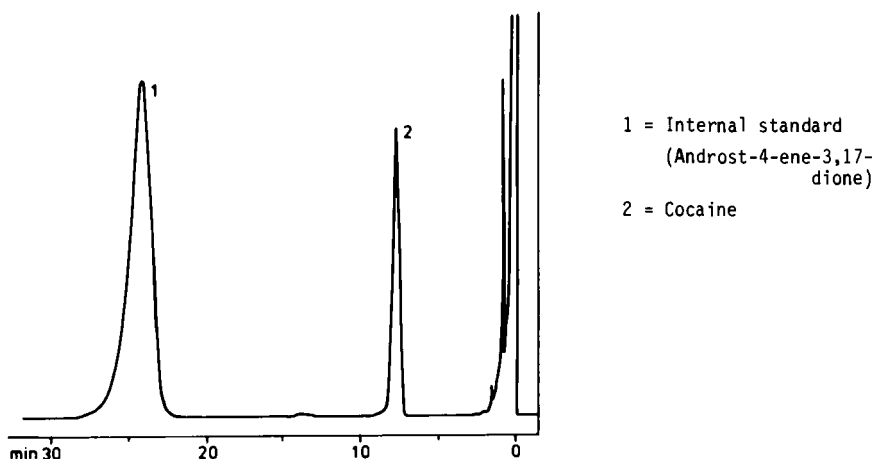
(*) Mean of triplicate determinations

(**) Mean coefficient of variation = 3.9

FIGURE 9.2

CHROMATOGRAM OF COCAINE FROM COCA LEAVES¹

Column: 2.4 m long by 2 mm I.D. packed glass column, 6 % OV-1 on Chromosorb W AWS at 220°C



Capillary gas chromatography of cocaine has so far been scarcely done. Christophersen and Rasmussen³⁶ analyzed a number of narcotic drugs, *i.a.* cocaine, on a glass capillary column coated with SE-30 using cold on-column injection (50°C). The oven temperature was programmed to 250°C with a speed of 5°C/min and cocaine was eluted at 198°C as a very sharp peak. The same injection technique was applied by Plotczyk³⁷ for a number of underivatized drugs, *i.a.* cocaine. He used fused silica capillary columns with non-extractable stationary phases (siloxane deactivated cross linked SE-54) and obtained excellent results.

TABLE 9.7

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF PSEUDOTROPINE ALKALIDS

Column	Solid support mesh	Stat.phase	%	Temperature	Comp.Preparation	Ref.
glass S, 2.4 m x 2 mm I.D.	CW AWS 100-200	OV-1	6	220°C	coc.qnt.pm.	1
glass, 6 ft x 4 mm I.D.	CW 80-100	SE-30	2-3	222°C	coc.	2
glass, 6 ft x 3 mm I.D.	CP ABS 100-140	SE-30	1.15	175°C	coc.	3
	+ PEG			200°C		
s.s., 5 ft x 1/8 in O.D.	CW AW 60-80	SE-30	5	210°C	coc.tox.id.	4
glass, 3 ft x 3 mm I.D.	CP AWS 80-100	SE-30	1	175°C		
-	-	XE-60	1	220°C		
-	-	EGSSY	1	230°C	alk.s.	5
-	-	HI-EFF 8B	1	230°C		
-	-	-		240°C		
glass, 6 ft x 1/4 in	GQ 100-120	SE-30	2.5	200°C	coc.bzecg.tox.	6
					ur.	
glass, 1 m	GP S 100-120	SE-52	2.5	150-250°C	alk.s.	7
		+ Ver.	1			
-	CW S 80-100	SE-52	2.5	150-250°C		

TABLE 9.7 (continued)

Column	Solid support	Stat.phase	%	Temperature	Comp.Prep.	Ref.
glass, 1 m	CW S 80-100	NGSE	1	150-200°C	alk.s.	7
glass, 4 ft x 4 mm I.D.	CG HP 80-100	OV-1	3	150°C	coc.(deriv.) id.ur.	8
glass, 4 ft x 4 mm I.D.	CW HP 100-120	OV-101	10	180-260°C pr 3°C/min	coc. ecq.	9
glass, 6 ft x 6 mm I.D.	GQ 100-120	OV-25	3	170-260°C pr 3°C/min	bzecg.	
glass 3 ft x 0.070 in I.D.	GP S 100-120	HI-EFF-8B	1	220-250°C	coc.tox.	10
glass, 3 ft x 2 mm	GQ S 100-120	NGS	1	230°C	alk.s.	11
-	GP AW 80-100	NGS	1	230°C		
glass, 6 ft x 2 mm I.D.	CW 80-100	+ PVP OV-17	7	250°C	coc.	12
glass, 2 ft x 1/4 in		SE-30	10	200-225°C	s.i. "caines"	13
glass, 1.8 m x 3.5 mm I.D.	CW S 80-100	SE-30	1	190°C		
-	CW S 100-120	Triton X	2	190°C	coc. + other	14
-	CW S 100-120	QF-1	3	195°C	drugs, pl.	
glass, 4 ft x 6 mm I.D.	CW HP 100-120	OV-1	3	190-280°C pr 200°C/min	cis-, trans- cinn.coc.	15
glass, 1.8 m x 4 mm I.D.	CW HP 100-120	OV-1	3	225°C	coc.qnt.	16,35
glass, 1 m x 3 mm I.D.	CW AWS 100-120	OV-17	2.9	185°C	coc.qnt.ur.	17,18
glass, 1 m x 3 mm I.D.	Sup 80-100	OV-17	3	220°C	coc.becg. der.	19
glass, 6 ft x 2 mm I.D.	CW HP 80-100	OV-17	3		qnt.ur.	
-	-	-	5	130-190°C	coc.ecg.bzecg.	20
glass, 10 ft	GQ 100-120	OV-225	5	110°C	qnt.ur.	
glass S, 5 ft	GQ	OV-225	2	190°C	coc. der.qnt.	21
glass, 1.8 m x 4 mm I.D.	GQ 100-120	OV-1	3	210°C	ur.	
glass, 3 ft x 2 mm I.D.	CW AWS 100-120	SP-2100-DB	3	200-230°C pr 30°C/min	coc.id.ur.	22
glass, 1.8 m x 2 mm I.D.	GQ 100-120	OV-17	3	260°C	coc. + other	24
glass, 1.8 m x 2 mm I.D.	Sup 80-100	OV-225	3	250°C	drugs, id.	
-	GQ 80-100	SE-30	3	200°C	coc.qnr.bl.pl.	25
-	Sup 80-100	OV-22	3	250°C	coc.qnt.pl.	27
glass S, 1.8 m x 2 mm I.D.	Sup 100-120	SP-2250-DA	3	230-260°C pr 12°C/min	becg.der.qnt.pl.	
-	-	SE-30	3	200-230°C pr 6°C/min	coc.bzecg.der.	28
glass S, 6 ft x 2 mm I.D.	GQ 80-100	UC W-98	3.8	200°C	qnt.ur.	29
glass S, 3 ft x 2 mm I.D.	CW HP 80-100	OV-17	3	200°C	coc.bzecg.der.	30
glass S, 1.8 m x 2 mm I.D.	GQ 100-200	OV-1	1.5	205°C	ur.	
glass, 74 cm x 2 mm I.D.	GQ AWS 100-120	OV-101	2	140-240°C pr 15°C/min	coc.bzecg.	31
glass, 1.8 m x 2 mm I.D.	CW HP 100-120	OV-17	3	255°C	qnt.ur.	33
glass cap. 20 m x 0.35 mm I.D.		SE-30		50-250°C pr 50°C/min	mecg.qnt.ur.	34
f.sil. cap. 25 m x 0.32 mm I.D.		SE-54		80-250°C pr 20°C/min	bzecg.der.qnt. ur.	36
					coc.	37

TABLE 9.8

PSEUDOTROPINE ALKALOIDS - LIST OF ABBREVIATIONS

ABS = acid, base washed, silanized	HP = high performance
alk = alkaloid	I.D. = inside diameter
AWS = acid washed, silanized	id = identification
bl = blood	mecg = methylecgonine
bzecg = benzoylecgonine	pl = plasma
CW = Chromosorb W	pm = plant material
cap = capillary	pr = (temperature) programming
CP = Chromosorb P	prep = pharmaceutical preparation
cinm.coc. = cinnamylcocaine	qnt = quantitative
der = derivative	S = silanized
ecg = ecgonine	s = separation
f.sil = fused silica	s.s = stainless steel
ft = feet	Sup = Supelcoport
GP = Gas Chrom P	tox = toxicology
GQ = Gas Chrom Q	Ver = Versamid 900
	ur = urine

TABLE 9.9

DERIVATIZATION OF SOME PSEUDOTROPINE ALKALOIDS

1 Silylation of ecgonine and benzoylecgonine in cocaine with N,O-bis(trimethylsilyl)acetamide

A 25 mg sample of cocaine hydrochloride (containing ecgonine and/or benzoylecgonine as impurities) is placed in a 1-ml glass-stoppered test tube and 500 μ l of the reagent is added. The tube is stoppered loosely and heated at 75°C for ten minutes with occasional agitation. After derivatization is complete, 3-4 μ l of the solution are injected for gas chromatographic analysis⁹.

2 Methylation of benzoylecgonine (to cocaine)

Methylating reagent: Add 1 volume of concentrated sulphuric acid slowly and with cooling, into 2 volumes of methanol.

To the purified residue obtained from the urine sample (5 ml) after extraction with 25 ml ethanol-chloroform (1:5), is added 0.6 ml of the methanolic sulphuric acid, the mixture is vortexed to ensure complete dissolution, and kept at 85°C for ten minutes. After cooling, the reaction product (cocaine) is extracted with diethyl ether. The diethyl ether is evaporized, 1 ml water and sufficient solid sodium bicarbonate for minimal neutralization added. The reaction product (cocaine) is extracted with 0.2 ml of chloroform, containing the internal standard (butylanthraquinone). Five μ l of the organic layer are injected for gas chromatographic analysis¹⁹.

3 Reduction of cocaine and/or benzoylecgonine with LiAlH_4 to 2-hydroxymethyl tropine and subsequent O-acylation with heptafluorobutyric anhydride or pentafluoropropionic anhydride.

To 5.0 ml of an aqueous solution containing cocaine is added 1 ml of saturated sodium tetraborate solution and 2.0 ml of cyclohexane. The drug is extracted into the cyclohexane phase and this transferred to a test tube. 50 μ l of a saturated LiAlH_4 solution in diethyl ether are added. After 3 minutes 50 μ l distilled water are added to the cyclohexane, and the mixture shaken. Next, 50 μ l of heptafluorobutyric anhydride (or alternatively pentafluoropropionic anhydride) are added to the cyclohexane phase and allowed to stand for 3-5 minutes

TABLE 9.9 (continued)

at room temperature. 2.0 ml of the cyclohexane solution are washed in 6 ml saturated sodium tetraborate solution - and the cyclohexane phase transferred to a clean tube, from which an aliquot can be taken for gas chromatographic-electron capture analysis⁸.

4 Acylation of ecgonine and benzoylecgonine with hexafluoroisopropanol-heptafluorobutyric anhydride (1:2).

To 0.05-1.0 µg of ecgonine and/or benzoylecgonine is added 100 µl of a mixture of hexafluoroisopropanol-heptafluorobutyric anhydride (1:2). After heating for 30 min at 75°C the excess of reagents is removed by evaporation. Then 1 ml of cyclohexane is added and 1 µl of the sample gas chromatographed²⁰.

5 Methylation of benzoylecgonine with diazomethane.

The sample of urine (1-5 ml) is first extracted with diethyl ether (3 x 5 ml) to remove cocaine, then with chloroform to extract benzoylecgonine. The chloroform extract is concentrated to 100 µl and treated with ethereal diazomethane (0.5 ml). Excess reagent is removed (40°C) after 5 minutes, the residue suspended in saturated sodium bicarbonate solution (1 ml), from which the cocaine is extracted with diethyl ether (2 x 1 ml). The diethyl ether extract is concentrated (50 µl) and 1-2 µl analyzed by GLC¹⁸.

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II.3 QUINOLINE ALKALOIDS

Chapter 10

CINCHONA ALKALOIDS

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10.1 CINCHONA ALKALOIDS

Quinine was gas chromatographed by Lloyd et al.¹ in 1960, when they demonstrated that high molecular weight alkaloids could be separated at relatively low temperatures on a 6 feet by 4 mm I.D. packed column with a 2-3 % SE-30 coated solid support. This stationary phase and other non-polar stationary phases have been used in a number of investigations, mainly with the purpose of investigating the gas chromatographic behaviour of alkaloids in general (Brochmann-Hanssen and Baerheim Svendsen², Smith et al.³, Sarsunová and Hrivňák⁴), and in pharmacological or toxicological analysis (Parker et al.⁵, Kazyak and Knoblock⁶, Street⁷, Moffat et al.⁸, Midha and Charette⁹). However, with non-polar stationary phases no separation of the diastereoisomers quinine and quinidine, as well as cinchonine and cinchonidine, can be achieved. Brochmann-Hanssen and Fontan^{10,11} in their systematic studies on gas chromatography of alkaloids with stationary phases of various polarity, found that the diastereoisomers quinine and quinidine, cinchonine and cinchonidine showed significant retention time differences on polar stationary phases.

In spite of these investigations, which showed new gas chromatographic possibilities, most workers continued using non-polar stationary phases in their analyses. A combination of thin-layer chromatography and gas chromatography on a non-polar stationary phase was used by Sarsunová and Hrivňák⁴ to achieve a separation of cinchonidine and quinidine, which could not be separated by thin-layer chromatography as quinine and cinchonine. Smith et al.³ applied gas chromatography of cinchona alkaloids as trimethyl silyl derivatives to detect alkaloidal impurities in pharmaceutical quinine and quinidine using an OV-225 3 % column. They were not able to separate the diastereoisomers, but they found that the dihydroanalogs were present in all 75 samples investigated, and the desmethoxyanalogs (cinchonine and cinchonidine) in about half of the samples. The relative retention times of the alkaloids investigated, as their trimethyl silyl derivatives, are given in Table 10.1.

Midha and Charette⁹ carried out quantitative determinations of quinidine in plasma and whole blood. Cinchonidine was added to the plasma sample to be analyzed as an internal standard. The alkaloids were extracted with benzene at pH 12.0. The residue from the extract was mixed with 25 μ l of trimethylanilinium hydroxide in methanol, and aliquots (1-2 μ l) were injected into the gas chromatograph in which the injection port was held at 350°C. The methyl derivatives of quinidine and the internal standard gave well separated symmetrical peaks. Detection by flame ionization gave a linear response over the range 0.2-12.0 μ g quinidine/ml plasma. The limit of detectability was 0.05 μ g/ml and the method was adequate for following blood profiles of 200 mg quinidine sulphate doses in humans. The recovery of quinidine

TABLE 10.1

RELATIVE RETENTION TIMES OF TRIMETHYLSILYL DERIVATIVES OF *CINCHONA* ALKALOIDS³

Glass column 6.1 m x 3 mm packed with 3 % OV-225 on Gas Chrom Q, temperature 225°C. t_R for quinidine approximately 21 min.

Alkaloid	Relative retention time
Cinchonidine	0.55
Cinchonine	0.55
Dihydrocinchonidine	0.50
Dihydrocinchonine	0.55
Epiquinidine	0.87
Epiquinine	0.95
Dihydroquinidine	0.93
Dihydroquinine	0.93
Quinidine	1.00
Quinine	1.00
Quininone	0.83
Quinotoxine (quinicine)	1.52
Thioglycerol adduct of quinidine	13.4

TABLE 10.2

RECOVERY OF QUINIDINE AND CINCHONIDINE FROM PLASMA DETERMINED BY GLC ASSAY⁹

Microgram added to 1 ml plasma	n	Mean microgram recovered	Mean percent recovery	Standard deviation of percent recovery
Quinidine 1.96	5	1.98	100.71	3.48
7.86	5	7.53	95.84	1.55
Mean 98.27 \pm 3.61 %				
Cinchonidine 2.11	7	1.72	81.63	2.84

TABLE 10.3

ESTIMATION OF QUINIDINE ADDED TO PLASMA BY GLC ASSAY⁹

Quinidine added μ g	n	Mean peak height	Standard deviation	CV, % *)
0.20	7	0.092	0.009	9.68
0.39	4	0.157	0.002	1.38
0.79	4	0.238	0.007	2.83
1.96	4	0.609	0.031	5.12
3.93	4	1.087	0.044	4.09
7.86	4	2.522	0.086	3.43
11.78	4	3.430	0.198	5.76

*) Mean CV = 4.61 %, $y = mx$ where $m = 0.295 \pm 0.008$; $r = 1$.

and cinchonidine from plasma determined by the gas chromatographic method is given in Table 10.2 and the estimation of quinidine added to plasma in Table 10.3.

In a paper published later, Midha et al.¹² described a comparison between a spectrofluorimetric method and the gas chromatographic technique published by Midha and Charette⁹, and they stated that the recovery of quinidine (98 % \pm 4 %) and the calibration curve from plasma by GLC were essentially identical to the ones reported earlier⁹. For a fast determination of

quinidine in plasma the authors found the GLC method preferable, as results can be available in 40 minutes.

Valentine et al.¹³ applied, in principle, the method of Midha and Charette⁹ for a quantitative assay of quinidines (quinidine and hydroquinidine) in plasma, using methylene chloride instead of benzene for the extraction of a small volume of plasma after basification. To the extract was added the internal standard, cinchonine; evaporation to dryness and reconstituting in a methanolic solution of trimethylanilinium hydroxide followed. An aliquot of this solution was analyzed by GLC via on-column methylation reaction. Evaluation of the method over the range 0.5 - 10 $\mu\text{g/ml}$ in human plasma gave a precision and accuracy overall of $\pm 4.5\%$ (RSD and RE). The plasma of several patients were analyzed by the GLC method as well as by a fluorimetric method for the levels of quinidine. Results from the two methods were comparable.

Moulin and Kinsun¹⁴ developed a simple, sensitive and accurate method for quinidine evaluation in serums, using chloroquine as an internal standard. One ml serum was made acid, extracted with hexane to remove lipids, made alkaline and again extracted, this time with diethyl ether:methanol (95:5) to isolate quinidine. From using an OV-1 3% column on Gas Chrom Q and a nitrogen detector, determinations down to 0.5 $\mu\text{g/ml}$ could be done, *i.e.* sufficient for therapeutic quinidine blood level determinations (1-5 $\mu\text{g/ml}$).

For a simultaneous quantitation of quinidine, procainamide and N-acetylprocainamide in serum Kessler et al.¹⁵ used a packed column, 1.83 m long by 2 mm I.D. with 3% OV-17 on Gas Chrom Q in combination with temperature programming from 230°C to 280°C. The dipropyl derivative of procainamide served as an internal standard. Good quantitative results were achieved with a nitrogen-phosphorus detector.

Although it has been demonstrated that quinine and quinidine only can be separated on packed columns with polar stationary phases^{10,11}, Furner et al.¹⁶ used a non-polar stationary phase - in combination with mass spectrometry - to achieve a gas chromatographic differentiation of the two isomers. When the mass spectrometer was operated in the selected ion monitoring (SIM) mode, 5 ng or less of quinine was detectable. That is, quinine and quinidine could be differentiated based upon selected ions. The possibility for developing a highly sensitive quantitative assay was discussed.

In a study on high temperature quantitative glass capillary gas chromatography of quinine/quinidine, Verzele et al.¹⁷ found that untreated soft glass gave better results than borosilicate glass. Some occasional tailing could be removed by the analysis of quinine/quinidine by sodium chloride dendrite deposition. With OV-1, OV-17, OV-225, Superox-4, RSL-802 and RSL-903 good peak shapes were obtained. The resolution of quinine and quinidine was zero on OV-1, but improved with increasing polarity of the stationary phases, as already stated by Brochmann-Hanssen and Fontan^{10,11}, and it was complete on RSL-903 (a highly polar polyaromatic sulfone), the most polar phase of the series. A 30 m by 0.3 mm I.D. sodium dendrite column coated with 0.15 μm layer of RSL-903 was used. A moving needle injector proved to be the best choice in order to obtain accurate quantitative results. Typical gas chromatograms are shown in Figure 2.2 and 2.3 (Chapter 2, Capillary columns).

The isothermal analysis was applied to the assay of quinine in soft drinks, and of quinine and quinidine in pharmaceutical preparations, with good results. By multiple analysis the standard deviation for quinine in soft drinks was found to be 1.97% and for quinine/quinidine in pharmaceutical preparations 1.07% and 0.90%, respectively.

Plotzcyk¹⁸ stated that fused silica capillary columns with non-extractable stationary

phases and cold on-column injection have given new possibilities in the gas chromatography of alkaloids. The inertness and high temperature stability of commercially available fused silica columns have eliminated the need for derivatization of many compounds, while providing enhanced sensitivity. With siloxane deactivated cross-linked and gum phase SE-54 fused silica columns excellent gas chromatographic results were obtained for a number of alkaloids, *i.a.* quinine. To improve polar solute peaks a binary solvent of 4 % methanol in toluene was used with cold on-column injection. The thermal lability of the underivatized alkaloid was minimized in splitless sampling by operating at the lowest inlet temperature possible. Plotczyk¹⁸ found that cold on-column injection yielded a linear response from 1 to 100 ng of drug with reproducibilities of 0.1-2 % at the 10 ng level.

TABLE 10.4

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF *CINCHONA* ALKALOIDS

Column	Solid support mesh	Stat.phase	%	Temperature	Comp. Prep.	Ref.
glass, 6 ft x 4 mm I.D.	CW 80-100	SE-30	2-3	222°C	alk. s.	1
glass, 6 ft x 3 mm I.D.	GP ABS 100-140 + PEG	SE-30	1.15	225°C	alk. s.	2
glass, 6.1 m x 3 mm	GQ	OV-225	3	225°C	alk.s.id., qnt. imp. qn.qnd.der.	3
glass, 6 ft x 4 mm I.D.	Ana ABS 100-120	SE-30	1	225°C	alk.tox.	6
-	-	QF-1	3	250°C		
s.s., 5 ft x 1/8 in O.D.	CW AW 60-80	SE-30	5	240°C	alk.tox.	5
glass, 80 cm x 1.5 mm I.D.	GP AW 80-100	OV-17	2	230°C	alk.qnt.	4
s.s., 6 ft x 1/8 in I.D.	CW AW 100-120	SE-30		235°C	alk.	7
glass, 1 m	CG AWS 80-100	OV-17	5	270°C		
glass, 2 m	-	SE-30	2		alk.	8
s.s., 1.2 m x 3 mm O.D.	CW AWS	OV-17		270°C	qnd.der.qnt.pl.	9
glass, 3 ft x 3 mm I.D.	GP AWS 80-100	SE-30	1	225°C		
-	-	XE-60	1	220°C		
-	-	EGSSY	1	230°C	alk. s.	10
-	-	HI-EFF 8B	1	230°C		
-	-	-		240°C		
glass, 3 ft x 3 mm I.D.	GQ S 80-100	NGS	1	230°C	alk. s.	11
-	GP AW 80-100	PVP	1	230°C		
-	-	+ NGS	1			
glass, 1.83 m x 2 mm I.D.	CW HP 80-100	OV-17	3	225-280°C	pr. qnd.hqnd der. qnt.pl.	13
s.s., 2 m x 2.17 mm	GQ 100-120	OV-1	3	270°C	qnd.qnt.bl.	14
glass, 1.83 m x 2 mm I.D.	GQ 100-120	OV-17	3	230-280°C	pr. qnd.qnt.pl.	15
glass S, 1.2 m x 2 mm I.D.	Sup. 100-200	SP-2250	3	16°C/min 220-270°C	pr. qn.qnd.GC-MS	16
glass cap. with sodium dendrite	30 m x 0.3 mm	RSL-903	0.15	180°C	alk. s.	17
f.sil. cap. 25 m x 0.32 mm I.D.		SE-54		75-260°C 10°C/min	pr. alk. s.	18

TABLE 10.5

CINCHONA ALKALOIDS - LIST OF ABBREVIATIONS

ABS = acid, base washed, silanized	id = identification
AW = acid washed	I.D. = inside diameter
alk = alkaloid	imp = impurity
Ana = Anakrom	O.D. = outside diameter
bl = blood	pl = plasma
cap = capillary	pm = plant material
CG = Chromosorb G	pr = (temperature) programming
CW = Chromosorb W	prep = pharmaceutical preparation
der = derivative	PVP = polyvinylpyrrolidone
diff = differentiation	qn = quinine
f.sil = fused silica	qnd = quinidine
ft = feet	qnt = quantitative
GP = Gas Chrom P	S = silanized
GQ = Gas Chrom Q	s = separation
HP = high performance	s.s = stainless steel
hqnd = hydroquinidine	Sup = Supelcoport
	tox = toxicology

TABLE 10.6

SILYLATION OF CINCHONA ALKALOIDS

1. With N-Methyl-N-trimethylsilyl-trifluoroacetamide

Add to 0.1 mg alkaloid in a 0.3 ml conial vial 0.1 ml N-methyl-N-trimethylsilyl-trifluoroacetamide or bistrimethylsilyl-trifluoroacetamide, cover with a Teflon-lined septum secured with a screw cap, and heat at 60°C for 45 minutes. Inject 3 μ l of the sample³.

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Chapter 11

ACRONYCHIA ALKALOIDS

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11.1 ACRONYCHIA ALKALOIDS

Gainer and Arnett¹ described a method for the quantitative determination of the antitumor alkaloid acronine. The alkaloid was extracted from various commonly used pharmaceutical excipients with chloroform. The recovery was good, as can be seen from Table 11.1. Cholesterol was used as an internal standard and a short packed column with OV-17 as stationary phase. A chromatogram is given in Figure 11.1 and the analysis of acronine in finished capsules in Table 11.2.

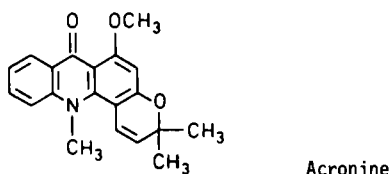


FIGURE 11.1

GAS CHROMATOGRAM OF ACRONINE/CHOLESTEROL¹

on a packed OV-17 column, 0.61 m by 3 mm I.D., with Diatoport S and 250°C

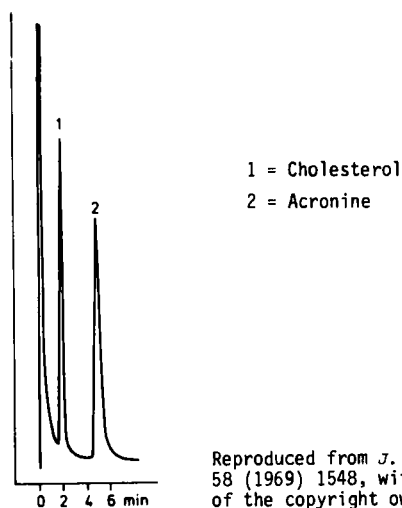


TABLE 11.1

RECOVERY OF ACRONINE FROM MIXES WITH VARIOUS EXCIPIENTS¹

Excipients	Acronine recovered, mg/g	Recovery %
Talc	1000.4	100.4
Stearic acid	508.3	101.7
Magnesium stearate	500.6	100.1
Silica gel	521.0	104.2
Tartaric acid	499.8	100.0
Ascorbic acid	503.8	100.8
Dipotassium phosphate	495.5	99.1
	426.1	85.2

TABLE 11.2

ACRONINE IN FINISHED CAPSULES¹

Content Wt., mg	Excipient	Acronine mg/capsule	n	Precision (RSD) %
170	Microcrystalline cellulose	24.9	5	± 1.63
290	Silica gel	23.0	5	± 1.17
300	Mg stearate and starch	23.7	4	± 2.77
300	Stearic acid and starch	24.8	5	± 1.16
300	Starch	25.3	5	± 1.74
380	Talc	25.5	5	± 1.59

Fong and Farnsworth² used gas chromatography on two packed columns (10 % UC-98 and 3.5 % SE-54) for the separation of nine alkaloids of *Acronychia baueri* (*Bauerella australiana*). The retention times relative to acronycidine on the UC-98 column and to melicopicine, on the SE-54 column are listed in Table 11.3.

TABLE 11.3

RELATIVE RETENTION TIMES OF *ACRONYCHIA* ALKALOIDS²

Alkaloid	Stationary phase	
	10 % UC-98	3.5 % SE-54
Alkaloid B	0.60	2.31
Normelicopine	0.60	2.05
Normelicopidine	0.61	
Melicopidine	0.63	
Acronycidine	1.00	
Melicopicine	2.06	
Normelicopicine	2.38	
Melicopine	3.75	
Acronycine	4.36	
Acronycidine time (min)	13.0	
Melicopicine time (min)		21.5

TABLE 11.4

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF *ACRONYCHIA* ALKALOIDS

Column	Solid support mesh	Stat.phase	%	Temperature	Comp.Preparation	Ref
glass S, 0.61 m x 3 mm I.D.	Dia S 80-100	OV-17		250°C	acr.qnt.prep.	1
s.s., 6 ft x 1/4 in O.D.	GQ 100-120	UC-98	10	260°C	alk.pm.	2
-	-	SE-54	3.5	230°C		

Abbreviations; Dia = Diatoport, S = silanized, s.s. = stainless steel, GQ = Gas Chrom Q,
acr. = acronine, qnt = quantitative, prep = pharmaceutical preparation,
pm = plant material

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II.4. PHENYLETHYLAMINE AND ISOQUINOLINE ALKALOIDS

Chapter 12

CACTUS ALKALOIDS

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12.1 CACTUS ALKALOIDS

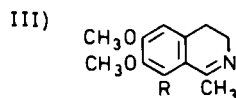
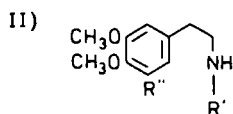
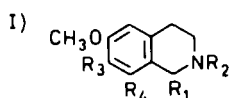
An investigation of the alkaloids of *Anhalonium lewinii* and some related compounds was carried out by Kapadia and Rao¹ using gas chromatography to study the structure-retention time relationship. A packed 1 % SE-52 column was used and the results are summarized in Table 12.1.

TABLE 12.1

GAS CHROMATOGRAPHY OF ANHALONIUM ALKALOIDS AND RELATED BASES¹

Column 12 feet long, I.D. 4 mm (glass); support Gas Chrom P 100-140 mesh, stationary phase SE-52 1 %; oven temperature 180°C. Compounds 6,8,10,12,14,17 and 18 are nonanhalonium bases. N = natural product from *Anhalonium lewinii*. S = prepared synthetically.

Compd.	I Tetrahydroisoquinolines	Substituents				Source	Mol. Wt.	t _R (min)
		R ₁	R ₂	R ₃	R ₄			
1	Anhalamine	H	H	OCH ₃	OH	N	208	4.82
2	Anhalidine	H	CH ₃	OCH ₃	OH	S	223	4.44
3	Anhalinine	H	H	OCH ₃	OCH ₃	S	223	4.16
4	Anhalonidine	CH ₃	H	OCH ₃	OH	N	223	4.41
5	Anhalonine	CH ₃	H	O-CH ₂	-O	N	221	5.20
6	Carnegine	CH ₃	CH ₃	OCH ₃	H	S	221	2.63
7	Lophophorine	CH ₃	CH ₃	O-CH ₂	-O	N	235	4.50
8	N-Methylanhalinine	H	CH ₃	OCH ₃	OCH ₃	S	237	3.09
9	O-Methylanhalonidine	CH ₃	H	OCH ₃	OCH ₃	S	237	3.56
10	O-Methylpellotine	CH ₃	CH ₃	OCH ₃	OCH ₃	S	251	3.00
11	Pellotine	CH ₃	CH ₃	OCH ₃	OH	N	237	3.94
12	Salsolidine	CH ₃	H	OCH ₃	H	S	207	2.96
II β-Phenylethylamines								
			R'		R''			
13	N-Acetylmescaline		O=C-CH ₃		OCH ₃	S	237	7.00
14	Homoveratrylamine		H		H	S	181	1.44
15	Mescaline		H		OCH ₃	N	211	3.38
16	N-Methylmescaline		CH ₃		OCH ₃	S	225	2.90
III Dihydroisoquinolines								
			R					
17	Schiff base A		H			S	205	3.28
18	Schiff base B		OCH ₃			S	235	4.26



The authors concluded that:

1. N-Monomethylation of primary and secondary amines, O-methylation, or C-monomethylation of the bases studied, resulted in a decrease in retention time. Although there was an increase in molecular weight corresponding to a methylene group, the observed decrease in retention times of the phenol ether and the alkylated amine could be rationalized as being due to the corresponding decrease in polarity of the derivatives, as compared to their parent compound. The decrease in retention of C-methyl derivative, as compared to its lower homolog, may be hypothesized as due to an interference of the methyl group in permitting the molecule to be adsorbed on the surface of the chromatographic column. The methyl group might sterically hinder the interaction of the polar phenolic hydroxyl and/or amine groups with the adsorbent.
2. Introduction of hydroxyl or methoxyl group, or an unsaturation in isoquinoline and methoxyl group in β -phenylethylamines, resulted in an increase in the retention time. It could again be conjectured that the increase in polarity of the derivatives caused the increase in adsorption on the liquid phase.
3. In tetrahydroisoquinolines, introduction of an hydroxyl group effected greater increase in retention than did a methoxyl group.
4. Replacement of two methoxyl groups with a methylenedioxy group in tetrahydroisoquinolines produced a noticeable increase in retention time.

The alkaloids of *Lophophora williamsii* (Lem. ex SD) Coult. belong to the phenylethylamine and the tetrahydroisoquinoline groups. Lundström and Agurell² carried out an investigation of them by means of gas chromatography on packed columns with stationary phases of various polarity. An SE-30 column was found to resolve the low molecular weight (> 200) phenylethylamines well, whereas an XE-60 column was very useful for the identification of the tetrahydroisoquinoline alkaloids. By means of semi-preparative columns, a better resolution of the alkaloid mixture was obtained than by means of the analytical columns. In Table 12.2 the gas chromatographic data for the peyote alkaloids and some related compounds are listed.

A trace alkaloid, 3,4-dimethoxyphenylethylamine was detected in peyote by means of combined gas chromatography/mass spectroscopy. A chromatogram of the phenolic and the non-phenolic alkaloids in peyote is given in Figure 12.1

For a screening of a series of cacti belonging mainly to *Cereus*, *Echinopsis*, *Helianthocereus* and *Trichocereus* for the presence or absence of alkaloids, Agurell³ adopted gas chromatography combined with mass spectroscopy, due to the great specificity and sensitivity thereby obtained. The following alkaloids were found:

N-Methyl-3,4-dimethoxyphenylethylamine	Mescaline
3,4-Dimethoxyphenylethylamine	Hordenine
3-Methoxytyramine	Tyramine
N-Methyltyramine	Macromerine
3,5-Dimethoxy-4-hydroxyphenylethylamine	Candicine
3,4-Dimethoxy-5-hydroxyphenylethylamine	Anhalonidine
	Trichocereine

Retention times of the reference compounds on two packed columns, one with 5 % SE-30 and one with 5 % XE-60, both operated at 150°C, are given in Figure 12.2.

TABLE 12.2

GLC DATA FOR PEYOTE ALKALOIDS AND RELATED COMPOUNDS ON PACKED COLUMNS OF VARIOUS POLARITY²

A,B and C: column 6 ft x 1/8 in O.D.; D and E: column 6 ft x 1/4 in O.D.; A = 5 % SE-30 on Gas Chrom P, 150°C; B = 7 % F 60 + 2 % Z on Gas Chrom P, 170°C; C = 5 % XE-60 on Chromosorb W, 150°C; D = 5 % SE-30 on Chromosorb W, 190°C; E = 5 % XE-60 on Chromosorb W, 184°C.

No	Alkaloid	Mol.Wt.	Retention time (min)				
			A	B	C	D	E
1	4-Methoxyphenylethylamine	151	1.5	2.1	0.9	1.0	0.7
2	Tyramine	137	1.8	-	4.6	1.2	3.5
3	N-Methyltyramine	151	2.2	-	4.4	1.3	3.3
4	Hordeanine	165	2.4	-	3.4	1.4	2.8
5	3,4-Dimethoxyphenylethylamine	181	3.5	5.4	2.8	1.8	2.6
6	Mescaline	211	6.8	11.1	6.6	3.3	5.2
7	N-Methylmescaline	225	8.0	11.3	6.2	3.2	5.0
8	O-Methylanhalidine	237	10.5	11.7	4.8	5.2	4.4
9	Anhalinine	223	11.3	17.5	7.8	5.6	6.8
10	O-Methylanhalonidine	237	11.6	14.5	6.1	5.3	5.5
11	Anhalidine	223	12.0	-	8.4	5.6	6.9
12	Anhalamine	209	12.4	-	13.6	5.8	10.5
13	Anhalonidine	223	13.0	-	10.8	6.1	8.2
14	Pellotine	237	13.1	-	7.6	6.0	6.5
15	Anhalonine	221	14.2	22.1	9.5	6.8	8.0
16	Lophophorine	235	14.5	18.3	6.7	6.2	6.3
17	Trichocereine	237	8.5	11.7	5.6	4.0	4.0

FIGURE 12.1

SEPARATION OF PHENOLIC (LEFT) AND NON-PHENOLIC (RIGHT) ALKALOIDS OF PEYOTE ON A 5 % PACKED XE-60 COLUMN AT 190°C².

Names of the alkaloids are in Table 12.3

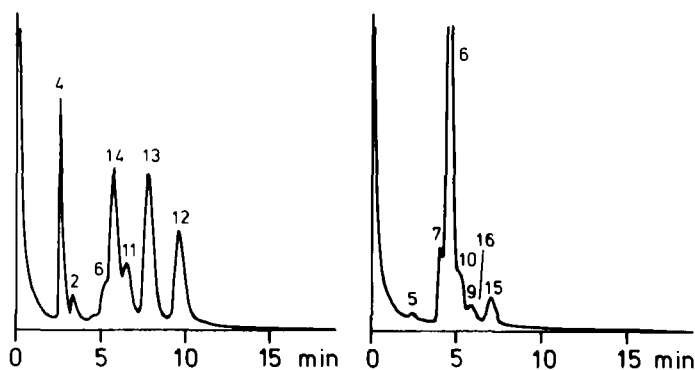


FIGURE 12.2

RETENTION TIMES OF REFERENCE COMPOUNDS ON TWO PACKED COLUMNS³

Both columns 5 ft x 1/8 in O.D.; 5 % SE-30 on Gas Chrom P and 5 % XE-60 on Chromosorb W, both at a column temperature of 150°C. No. and names of compounds as in Table 12.3.

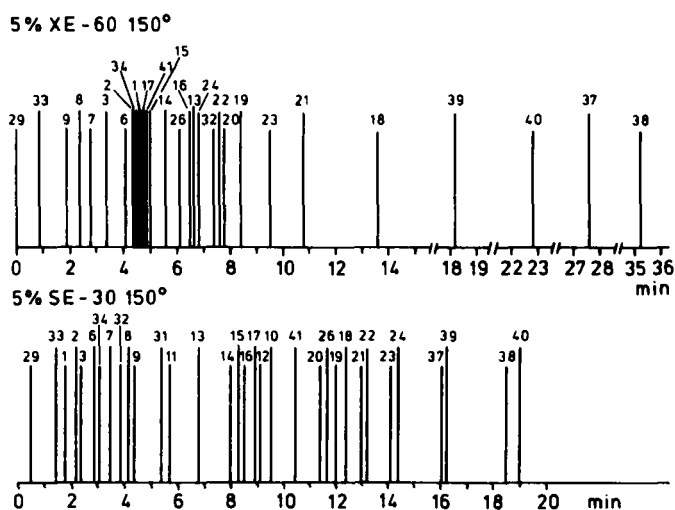


TABLE 12.3

CACTUS ALKALOIDS AND COMPOUNDS RELATED TO KNOWN CACTUS ALKALOIDS³

1 Tyramine	26 O-Methylanhalonidine
2 N-Methyltyramine	27 Lophocerine
3 Hordenine	28 Pilocerpine
4 N-Methyl-4-methoxyphenylethylamine	29 Phenylethylamine
5 Dopamine	30 Octopamine
6 3-Methoxy-4-hydroxyphenylethylamine	31 Oxedrine
7 3,4-Dimethoxyphenylethylamine	32 β-O-Methyloxedrine
8 N-Methyl-3,4-dimethoxyphenylethylamine	33 O-Methyltyramine
9 N,N-Dimethyl-3,4-dimethoxyphenylethylamine	34 4-Methoxy-3-hydroxyphenylethylamine
10 Macromerine	35 N-Methyl-4-hydroxy-3-methoxyphenylethylamine
11 3,4-Dimethoxy-5-hydroxyphenylethylamine	36 N,N-Dimethyl-4-hydroxy-3-methoxyphenylethylamine
12 3,5-Dimethoxy-4-hydroxyphenylethylamine	37 <i>cis</i> -1-Methyl-4-hydroxy-6,7-dimethoxytetrahydroisoquinoline
13 Mescaline	38 <i>trans</i> -1-Methyl-4-hydroxy-6,7-dimethoxytetrahydroisoquinoline
14 N-Methylmescaline	39 <i>cis</i> -1,2-Dimethyl-4-hydroxy-6,7-dimethoxytetrahydroisoquinoline
15 Trichocereine	40 <i>trans</i> -1,2-Dimethyl-4-hydroxy-6,7-dimethoxytetrahydroisoquinoline
16 Norcarnegine	41 O-Methylanhalidine
17 Carnegine	42 O,N-Dimethylanhalonidine
18 Anhalamine	
19 Anhalidine	
20 Anhalinine	
21 Anhalonidine	
22 Pellotine	
23 Anhalonine	
24 Lophophorine	
25 Peyophorine	

Doetsch et al.⁴ used gas chromatography for the separation of the non-phenolic alkaloids and for the quantitative determination of mescaline in *Pereskia*, *Pereskopsis* and *Islaya* species. A packed 1.5 % OV-101 on Chromosorb G column and a column temperature of 150°C was used for the analysis.

The amount of mescaline in the plasma of rabbits after intravenous injection was determined by Van Peteghem et al.⁵. Mescaline was converted to the trifluoroacetyl derivative and trifluoroacetyl-²H₂-mescaline was used as an internal standard. The method that was developed combined the specificity of gas chromatographic retention times and mass spectral fragmentation pattern with the sensitivity of the mass fragmentographic detection. Plasma samples of 0.5 ml were used. 200 ng of the internal standard were added to the sample, the pH adjusted to pH 10 and the extraction performed with benzene. The benzene extract was evaporated to dryness, the residue dissolved in 0.2 ml ethyl acetate and 0.2 ml of trifluoroacetic acid anhydride. After heating for 30 minutes at 60°C, the solvent was evaporated and the residue dissolved in 10-20 µl of methanol, and 1-2 µl injected for gas chromatographic analysis. The fragmentographic detection allowed detection down to 5 ng/ml and the relative standard deviation was 5 %.

TABLE 12.4

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF CACTUS ALKALOIDS

Column	Solid support	Stat.phase	%	Temperature	Comp.Prep.	Ref
glass, 12 ft x 4 mm I.D.	GP 100-140	SE-52	1	180°C	Anh.alk.	1
glass, 6 ft x 1/4 in O.D.	GP 100-120	SE-30	5	150°C	peyot.alk.	2
-	CW AWS 80-100	XE-60	5	150°C		
-	GP AWS 100-120	F 60	7	170°C		
glass, 5 ft x 1/8 in	GP AWS CW AWS	+ Z	2	170°C	cact.alk.	3
		SE-30	5			
		XE-60	5			
s.s., 2.5 m x 3.2 mm	CG 100-120	OV-101	1.5	150°C	cact.alk.	4
glass S, 1.5 m x 6.3 mm	Var 100-120	QF-1	2.5	195°C	msc.qnt.pl.	5

Abbreviations: alk = alkaloid, Anh = Anhalonium, AWS = acid washed, silanized, cact = cactus, comp = compound, CW = Chromosorb W, GP = Gas Chrom P, I.D. = inside diameter, in = inch, msc = mescaline, O.D. = outside diameter, peyot = peyote, pl = plasma, prep = pharmaceutical preparation, qnt = quantitative, S = silanized, s.s. = stainless steel, Var = Varaport.

12.3 REFERENCES

- 1 G.J. Kapadia and G.S. Rao, *J. Pharm. Sci.*, 54 (1965) 1817.
- 2 J. Lundström and S. Agurell, *J. Chromatogr.*, 36 (1968) 105.
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- 4 P. W. Doetsch, J.M. Cassidy and J.L. McLaughlin, *J. Chromatogr.*, 168 (1980) 79.
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Chapter 13

EPHEDRA ALKALOIDS

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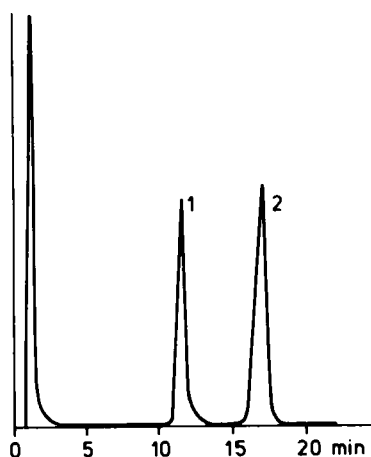
13.1 *EPHEDRA* ALKALOIDS

Ephedrine and its isomer pseudoephedrine are alkaloids in *Ephedra species*. Brochmann-Hansen and Baerheim Svendsen¹ reported the first gas chromatographic separation of these two alkaloids in a study on the separation and identification of 11 sympathomimetic amines on a 1.15 % SE-30 packed column at 104°C. However, ephedrine and pseudoephedrine could not be separated as such, but were separated as their oxazolidine derivatives after treatment with acetone. A typical chromatogram is given in Tale 13.1.

FIGURE 13.1

GAS CHROMATOGRAM OF EPHEDRINE (1) AND PSEUDOEPHEDRINE (2)¹

after 3 hours at room temperature in acetone solution. Column: 1.15 % SE-30 on Gas Chrom P at 104°C.



1 = Ephedrine

2 = Pseudoephedrine

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Parker et al.² applied gas chromatography for the separation and identification of some sympathomimetic amines. They used a packed column of 5 % Carbowax 20 M on firebrick treated with 5 % potassium hydroxide, at a column temperature of 170°C or 190°C. Ephedrine and pseudoephedrine were not separated, however.

In a couple of papers, Beckett and Testa^{3,4} described the gas chromatographic separation

of the optical isomers of "ephedrines" and "pseudoephedrines" as their N-trifluoroacetyl-L-propyl derivatives. For (+)ephedrine and (-)ephedrine a usable resolution was obtained (resolution factor 0.78), but for (+)pseudoephedrine and (-)pseudoephedrine the resolution factor was poor (resolution factor 0.45).

Gilbert and Brooks⁵ succeeded in separating the 4 diastereoisomeric ephedrines as their N-acetyl-O-trimethylsilyl derivatives. For the separation of enantiomers, conversion into N-(R)- α -phenylbutyryl-O-trimethylsilyl derivative was effective for ephedrine and pseudoephedrine, but not for that of nor-ephedrine. The retention indices of diastereomeric ephedrines as their N-acetyl-O-trimethylsilyl ether derivatives are given in Table 13.1 and the retention indices of ephedrines as diastereomeric N-(R)- α -phenylbutyryl-O-trimethylsilyl ether derivatives are in Table 13.2. A gas chromatogram of the separation of nor-pseudoephedrine, ephedrine and pseudoephedrine as their N-acetyl-O-trimethylsilyl ether derivatives, is given in Figure 13.1.

FIGURE 13.2

GAS CHROMATOGRAM OF EPHEDRINES⁵

on a 5 m by 3 mm I.D. packed glass column with 1 % OV-17 on Gas Chrom Q at 170°C; 1 = Nor-pseudoephedrine, 2 = Ephedrine, 3 = Pseudoephedrine as their N-acetyl-O-trimethylsilylether derivatives.

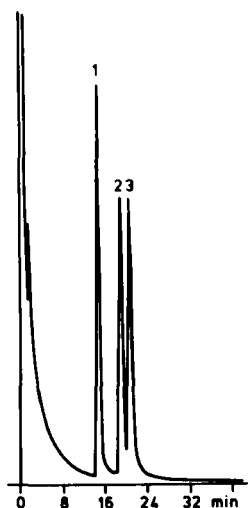


TABLE 13.1

RETENTION INDICES OF DIASTEREOMERIC EPHEDRINES⁵

as N-acetyl-O-trimethylsilyl ether derivatives

Compound	Configuration	Retention index, 5 m 1 % OV-17, 170°C
(-)-Ephedrine	1R,2S	1925
(-)-Pseudoephedrine	1R,2R	1945
(+)-Nor-ephedrine	1S,2R	1865
(-)-Nor-pseudoephedrine	1R,2R	1870

TABLE 13.2

RETENTION INDICES OF EPHEDRINES⁵as diastereomeric N-(R)- α -phenylbutyryl-O-trimethylsilyl ether derivatives

Parent compound	Configuration	Retention indices	
		1 % OV-1 (190°C)	1 % OV-17 (210°C)
(+)-Ephedrine	1S,2R	2300	2555
(-)-Ephedrine	1R,2S	2310	2570
(+)-Pseudoephedrine *	1S,2S	2290	2570
(-)-Pseudoephedrine	1R,2R	2325	2600
(+)-Nor-ephedrine	1S,2R	2280	2550
(-)-Nor-ephedrine *	1R,2S	2280	2550
(+)-Nor-pseudoephedrine *	1S,2S	2240	2520
(-)-Nor-pseudoephedrine	1R,2R	2260	2545

* Data obtained from (\pm) sample

13.2.1. Ephedrine in plant material

Yamasaki et al.⁶ applied gas chromatography to the separation and quantitative analysis of the alkaloids in some *Ephedra* species collected around the Himalayas. A satisfactory separation of the alkaloids and quantitative determination of L-ephedrine and D-pseudoephedrine was achieved using oxazolidine formation with acetone¹.

13.2.2. Ephedrine in pharmaceutical preparations

In order to determine ephedrine in aerosols, Lawless et al.⁷ expired the aerosol through a piece of stainless-steel tubing into a 10 ml volumetric flask containing acetone. The volumetric flask was brought to volume and an aliquot injected for the gas chromatographic analysis on a 1.15 % SE-30 packed column at 171°C. Over 99.5 % of the amount of ephedrine present in the aerosol could be determined by this method.

For the determination of ephedrine in tablets also containing phenobarbital and theophylline, Elefant et al.⁸ converted ephedrine to benzaldehyde by periodate oxydation prior to gas chromatography. Using benzyl alcohol as an internal standard and a packed column of 3 % HI-EFF 8 BP on Gas Chrom Q and temperature programming from 80°C to 130°C, good results were obtained. The standard deviation for 10 individual tablets declared to contain 24 mg ephedrine was found to be 1.1 %.

The same method was also applied by Vuorinen and Halmenkoski⁹. For tablets containing 25 mg and 22 mg ephedrine hydrochloride, a recovery of 99.98 % and 99.96 % was found, with a standard deviation of ± 1.25 % and ± 0.81 %, respectively. An 5 % Carbowax 4000 packed column on Chromosorb W was used.

Iconomou et al.¹⁰ determined the ephedrine content in cough syrup and extracted it with chloroform after basification of the syrup with ammonia. The chloroform extract was evaporated and the residue dissolved in a chloroform solution containing the internal standard, diphenyl. Chromatograms of ephedrine-diphenyl solutions (0.1 μ g/ μ l diphenyl) on a 2 m long packed 2 % Versamide 900 on Chromosorb W column with 5 % KOH at 175°C are given in Figure 13.3.

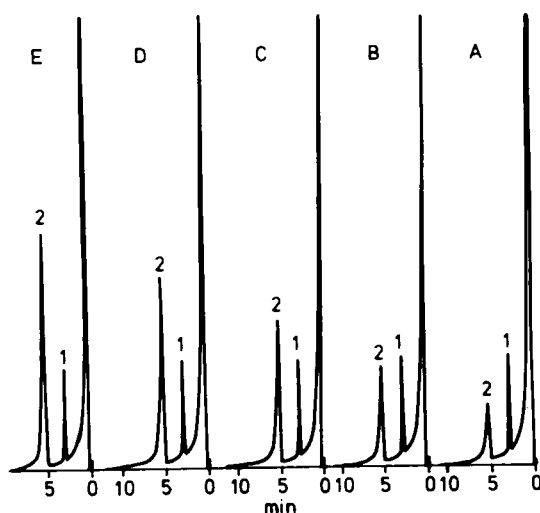
The "Joint Committee" of the Pharmaceutical Society and the Society for Analytical Chemistry¹¹ recommended a gas chromatographic method for the determination of ephedrine in tablets, elixir and nasal drops, using phenmetrazine as an internal standard. Ephedrine was extracted

from aqueous solutions of the preparations by means of diethyl ether after basification with sodium hydroxide. The diethyl ether extract was concentrated and used for the assay on a 2 % Carbowax 6000 packed column on Chromosorb G impregnated with 5 % sodium hydroxide. Coefficients of variations within laboratories were not greater than 3 % for tablets containing 30 mg ephedrine.

FIGURE 13.3

CHROMATOGRAMS OF SOLUTIONS OF EPHEDRINE¹⁰

1 = Diphenyl (internal standard) (0.1 $\mu\text{g}/\mu\text{l}$) and 2 = Ephedrine: A = 0.5 $\mu\text{g}/\mu\text{l}$, B = 0.75 $\mu\text{g}/\mu\text{l}$, C = 1.0 $\mu\text{g}/\mu\text{l}$, D = 1.25 $\mu\text{g}/\mu\text{l}$, E = 1.50 $\mu\text{g}/\mu\text{l}$. A packed column with 2 % Versamide 900 on Chromosorb W + 5 % KOH at 175°C.



13.2.3. Ephedrine in biological fluids

Urine

Beckett and Wilkinson¹² developed a method for the identification and estimation of ephedrine and its congeners in urine. Urine samples of 1-5 ml were acidified and extracted with diethyl ether to remove neutral and acidic compounds. The urine was then made alkaline and the amines were extracted with diethyl ether. This extract was used after concentration for gas chromatographic analysis. Because ephedrine and pseudoephedrine could not be separated as such, their acetone derivatives were prepared¹. The retention times of some ephedrine congeners are given in Table 13.3.

Welling et al.¹³ used gas chromatography for the determination of ephedrine in urine. Samples of 5 ml urine were acidified and extracted with diethyl ether; the diethyl ether was discarded. After basification with sodium hydroxide the sample was extracted again with

diethyl ether. This extract was used for the gas chromatographic analysis; 4-aminoacetophenone was used as an internal standard. The internal standard was added to the urine sample prior to the alkaline extraction. A typical chromatogram is given in Figure 13.4.

FIGURE 13.4

GAS CHROMATOGRAM OF EPHEDRINE FROM URINE EXTRACT¹³

on a 3 % OV-1 packed column on Gas Chrom Q, 1.83 m by 4 mm I.D., at 140°C. A = extract of blank urine spiked with ephedrine sulphate (12 µg/ml) and 4-aminoacetophenone (5 µg/ml); B and C = gas chromatograms of a 0.5-1.0 hr and a 6-9 hr urine sample of a subject having received 25 mg of ephedrine sulphate. 1 = ephedrine, 2 = 4-aminoacetophenone, 3 = norephedrine.

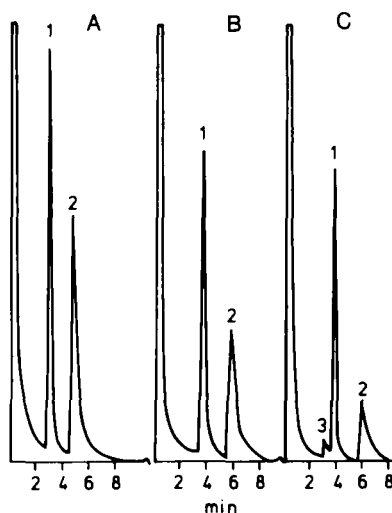


TABLE 13.3

RETENTION TIMES OF SOME EPHEDRINE CONGENERS¹²

on a 2 % PEG 6000 + 5 % KOH packed column on Chromosorb G, 1 m by 1/8 inch O.D., at 165°C

Compound	Retention time (min)	
	Base	Acetone derivative
Internal marker (2,6-dimethylphenoxy)ethylamine	4.9	8.0
Methylephedrine	6.8	not formed
Pseudoephedrine	8.2	3.6
Ephedrine	8.2	4.0
Nor-pseudoephedrine	10.5	4.2
Nor-ephedrine	11.3	4.0

Plasma

Pickup and Paterson¹⁴ developed a method for the determination of ephedrine in plasma. To plasma samples of 3 ml the internal standard (phendimetrazine) and alkali were added prior to extraction with diethyl ether. The diethyl ether extract was concentrated to about 20 µl

and 4 μ l injected for the gas chromatographic assay on a 8 % Carbowax 20 M on Chromosorb W column at 180°C. Accuracy and precision of the method was good. With the assay of twelve plasma samples containing 25 ng ephedrine base per ml, the coefficient of variation was 9.7 %.

TABLE 13.4

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF *EPHEDRA* ALKALOIDS

Column	Solid support mesh	Stat.phase	%	Temperature	Comp.Prep.	Ref.
glass, 6-8 ft x 3 mm I.D.	GP AWS 100-140	SE-30	1.15	104°C	ep.psep.s.	1
-	CW AWS 60-80	SE-30	1.15	104°C		
glass, 4 ft x 5 mm I.D.	Fib 100-120	Cab 20M	5	170-190°C	ep.psep.s.	2
		+ KOH	5			
s.s., 2 m x 1/8 in O.D.	CG AWS 100-120	SE-30	3	170°C	"eps", "pseps"	3,4
glass S, 3 m x 3 mm I.D.	GQ 100-120	OV-1	1		diast.enant.eps.	5
glass S, 5 m x 3 mm I.D.		OV-17	1			
s.s., 6-8 ft x 3 mm I.D.	CP 100-140	SE-30	1.15	171°C	ep.qnt.aeros.	7
glass, 6 ft x 0.25 in O.D.	GQ 100-120	HI-EFF 8B	3	80-130°C pr 6°C/min	ep.qnt.bn2ld.	8
s.s., 10 ft x 1/8 in I.D.	CW 80-100	Cab 4000	5	140°C	ep.qnt.bzld.	9
glass, 2 m x 2.5 mm I.D.	GP S 80-100	SE-30	3	145°C	ep.qnt.syr.	10
-	CW 80-100	Ver	2	175°C		
		+ KOH	5			
glass, 1 m x 4 mm I.D.	CG AWS 80-100	Cab 6000	2	150°C	ep.qnt.prep.	11
s.s.,		+ KOH	5			
s.s., 1 m x 1/8 in O.D.	CG AWS 80-100	PEG 6000	2	165°C	ep.cg.qnt.ur.	12
		+ KOH	5			
1.83 m x 4 mm I.D.	GQ 100-120	OV-1	3	140°C	ep.qnt.ur.	13
glass, 2 m x 1/4 in O.D.	CW 80-100	Cab 20 M	8	180°C	ep.qnt.pl.	14
		+ KOH	2			

TABLE 13.5

EPHEDRA ALKALOIDS - LIST OF ABBREVIATIONS

aeros = aerosol
 AWS = acid washed, silanized
 bzld = benzaldehyde
 Cab = Carbowax
 cg = congener
 CG = Chromosorb G
 comp = compound
 CW = Chromosorb W
 diast = diastereomeric
 enant = enantiomeric
 ep = ephedrine
 "eps" = "ephedrine"
 Fib = firebrick
 ft = feet

GP = Gas Chrom P
 GQ = Gas Chrom Q
 I.D. = inside diameter
 in = inch
 O.D. = outside diameter
 psep = pseudoephedrine
 "pseps" = "pseudoephedrines"
 pl = plasma
 prep = pharmaceutical preparation
 qnt = quantitative
 s = separation
 S = silanized
 s.s. = stainless steel
 syr = syrup
 ur = urine

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Chapter 14

OPIUM ALKALOIDS

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14.1. OPIUM ALKALOIDS

14.1.1. Packed columns

The opium alkaloids have engaged the interest of scientists since the isolation of morphine by Sertürner in 1806. The isolation, characterization and quantification of these alkaloids have been a continuing challenge. Gas chromatography of opium alkaloids has been performed *i.a.* for the analysis of the alkaloids present in the crude drug itself, especially for the quantitative determination of morphine, as well as for the analysis of opium alkaloids, mainly morphine - and heroin - in biological materials. Most studies have so far been carried out with packed columns, only a limited number with capillary columns.

Gas chromatography of opium alkaloids, metabolites and congeners has mostly been carried out on non-polar or slightly polar stationary phases, such as SE-30, SE-52, OV-17, OV-1, QF-1, and XE-60. In some cases, however, more polar stationary phases have been employed because of their greater selectivity (PEG 20 M, DEGS, EGSS-Y, NGS and HI-EFF 8B). However, the retention times of the alkaloids are significantly increased on very polar stationary phases, which for high molecular weight alkaloids may lead to very high, and for practical use often undesirably high, retention times, even when working with columns with a low concentration of stationary phase.

To avoid adsorption due to "active sites" on the solid support when it is coated with a low percentage of stationary liquid, an acid/base washed and silanized support is mostly used. Brochmann-Hanssen and Furry¹ also coated the solid support with polyethylene glycol 4000 and nonylphenoxyethyleneoxyethanol, 0.05 % of each, before coating with 2 % SE-30. Street² used a deactivation procedure for solid support, whereby a water saturated solution of the stationary phase (SE-52) in toluene was used, followed by heating of the product in an oxygen-free nitrogen stream at 370°C for 18 hours. Later Street et al.³ showed that acylation of the solid support (diatomaceous earth) with benzoylchloride in pyridine prior to coating with a silicone stationary phase and followed by heating in an atmosphere of nitrogen, led to gas chromatographic columns with a marked reduction in adsorption - even for unmodified polar compounds such as morphine.

14.1.1.1. Separation and identification

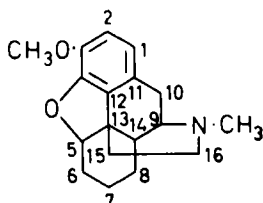
In their first paper on gas chromatography of alkaloids, Lloyd et al.⁴ chromatographed the main opium alkaloids on a SE-30 column. Eddy et al.⁵ made use of the same stationary phase in studies on the relationship between the relative amount of the main opium alkaloids and the origin of opium. They investigated a number of authentic UN opium samples on a 1 % SE-30 column on Gas Chrom P with temperature programming and concluded that gas chromatographic analysis immediately establishes a characteristic fingerprint of the individual alkaloid sample and thus promises to aid in the correlation between source country and sample.

Yamaguchi et al.⁶ gas chromatographed more than 40 opium alkaloids and related compounds to determine whether or not a correlation existed between the retention time and the chemical structure (Table 14.1).

TABLE 14.1

RELATIVE RETENTION TIMES OF CODEINE AND RELATED ALKALOIDS⁶

Column: 6 ft long by 8 mm, 1 % SE-30 on Gas Chrom P (100-140 mesh) at 185°C. Codeine, t_R 4.71 min



Dihydrodesoxycodeine D

Compound	t_R (rel)	Characteristics
1 Desoxycodeine E	0.64	$\Delta_{7,8}$
2 Dihydrodesoxycodeine D	0.66	
3 Isocodeine	0.74	$\Delta_{7,8}$, $C_6\beta$ -OH
4 Tetrahydrodesoxycodeine	0.77	Opening the ether ring, C_4 -OH
5 Pseudocodeinone	0.80	$\Delta_{6,7}$, $C_6=O$
6 14-Hydroxydihydrodesoxycodeine	0.84	$C_{14}\beta$ -OH
7 Dihydrodesoxythebainone	0.86	Opening the ether ring C_6 -O, C_4 desoxy
8 Allo pseudocodeine	0.91	$\Delta_{6,7}$, $C_6\alpha$ -OH
9 14-Hydroxydesoxycodeine E	0.91	$\Delta_{7,8}$, $C_{14}\beta$ -OH
10 14-Acetyldihydrodesoxycodeine D	0.93	$C_{14}\beta$ -OAc
11 Dihydrocodeine	0.96	$C_6\alpha$ -OH
12 Neopine	0.97	$\Delta_{8,14}$, $C_6\alpha$ -OH
13 Codeine methyl ether	0.98	$\Delta_{7,8}$, $C_6\alpha$ -OCH ₃
14 Codeine	1.00	$\Delta_{7,8}$, $C_6\alpha$ -OH
15 Pseudocodeine	1.03	$\Delta_{7,8}$, $C_6\beta$ -OH
16 Dihydro-6 α -thebainol methyl ether	1.07	Opening the ether ring, C_4 -OCH ₃ , $C_6\alpha$ -OH
17 Dihydroisocodeine	1.07	$C_6\beta$ -OH
18 Dionine (ethyl morphine)	1.09	$\Delta_{6,7}$, C_3 -OC ₂ H ₅ , $C_6\alpha$ -OH
19 Norcodeine	1.09	$\Delta_{7,8}$, $C_3\alpha$ -OH, N-H
20 14-Hydroxytetrahydrodesoxycodeine	1.13	Opening the ether ring, C_4 -OH, $C_{11}\beta$ -OH
21 Dihydrocodeinone	1.15	$C_6=O$
22 Morphine	1.17	$\Delta_{7,8}$, C_3 -OH, $C_6\alpha$ -OH
23 Dihydro-6 β -thebainol methyl ether	1.20	Opening the ether ring, C_4 -OCH ₃ , $C_6\beta$ -OH
24 Dihydrothebainone methyl ether	1.22	Opening the ether ring, C_4 -OCH ₃ , $C_6=O$
25 14-Hydroxydihydrodesoxycodeine C	1.30	Opening the ether ring, $\Delta_{5,6}$, C_4 -OH, $C_{14}\beta$ -OH

TABLE 14.1 (continued)

Compound	t_R (rel)	Characteristics
26 14-Hydroxydihydrocodeine	1.35	$C_6\alpha$ -OH, $C_{14}\beta$ -OH
27 14-Hydroxycodeine	1.35	Δ_7^8 , $C_6\alpha$ -OH, $C_{14}\beta$ -OH
28 8,14-Dihydroxydihydrocodeinone	1.37	$C_6\alpha$, $C_8\beta$ -OH, $C_{14}\beta$ -OH
29 14-Hydroxycodeinone	1.38	Δ_7^8 , $C_8=O$, $C_{14}\beta$ -OH
30 14-Hydroxydihydrocodeinone	1.39	$C_6\alpha$, $C_{14}\beta$ -OH
31 14-Acetylcocodeinone	1.41	Δ_7^8 , $C_6=O$, $C_{14}\beta$ -OAc
32 14-Hydroxydihydroisocodeine	1.43	$C_6\beta$, $C_{14}\beta$ -OH
33 Thebaine	1.48	Δ_6^7 , Δ_8^{14} , C_6 -OCH ₃
34 8-Methoxy-14-hydroxydihydrocodeinone	1.48	$C_6\alpha$, $C_8\beta$ -OCH ₃ , $C_{14}\beta$ -OH
35 Dihydrothebainone ϕ	1.52	Opening the ether ring, Δ_5^6 , Δ_8^{14} , C_4 -OH, C_6 -OCH ₃ , $C_6=O$, $C_8\beta$ -OAc
36 14-Acetyldihydrocodeinone	1.52	
37 Dihydrothebainone	1.61	Opening the ether ring, C_4 -OH, $C_6=O$
38 N-Propargyl-14-hydroxydihydronorcodeinone	1.69	$C_6=O$, $C_{14}\beta$ -OH, N-CH ₂ C \equiv CH
39 Sinomenine methyl ether	1.87	Antipode, opening the ether ring, Δ_7^8 , C_4 -OCH ₃ , $C_6=O$, C_7 -OCH ₃
40 14-Hydroxydihydronorcodeinone	1.91	$C_6\alpha$, $C_{14}\beta$ -OH, N \equiv H
41 Dihydrosinomenine	2.00	Antipode, opening the ether ring, C_4 -OH, $C_6=O$, C_7 -OCH ₃
42 14-Hydroxydihydrothebainone	2.45	Opening the ether ring, C_4 -OH, $C_6=O$, $C_{14}\beta$ -OH
43 Sinomenine	2.83	Antipode, opening the ether ring, C_4 -OH, $C_6=O$, C_7 -OCH ₃ , Δ_7^8

For the application of gas chromatography to toxicological analysis, Parker et al.⁷ studied a series of alkaloids including the main opium alkaloids and some related compounds, using a SE-30 column; so also did Kazyak and Knoblock⁸. In a study on the systematic application of gas chromatography to toxicological analysis, Jain and Kirk⁹ utilized the very polar HI-EFF-8B (1 %) as stationary phase for a number of opium alkaloids and related compounds: Codeine, dihydrocodeine, dihydrohydroxycodeinone, heroin, morphine, noscapine, papaverine and thebaine.

For the identification of a number of opium alkaloids (morphine, codeine, thebaine, noscapine, papaverine, cryptopine, narceine) and heroin, 3-O-acetylmorphine, 6-O-acetylmorphine and acetylcodeine, Viala et al.¹⁰ made use of three gas chromatographic columns of different polarities (SE-30 3 %, OV-1 2 % and OV-1 2 % plus Igepal 0.2 %). They obtained reliable separation and identification in this way, which was based on the difference in the retention times in the three columns.

To achieve a best possible identification based on retention times, Moffat et al.¹¹ calculated the retention indices for a number of basic drugs, including opium alkaloids and congeners, on eight stationary phases of various polarity. They concluded that low-polarity columns, such as SE-30 or OV-17, should be preferred for the identification of basic drugs with gas chromatography.

Later Moffat¹² calculated the retention indices for a series of opium alkaloids and congeners on a SE-30 column (Table 14.2).

Liras¹³ used an OV-17 column for investigations on the metabolism of morphine and codeine by *Arthrobacter* species, and gave the gas chromatographic parameters of eight oxidized compounds, found as metabolites (14-hydroxymorphine, 14-hydroxymorphinone, 14-hydroxydihydromorphinone, dihydromorphinone, codeinone, 14-hydroxycodeine, 14-hydroxycodeinone and 14-hydroxydihydrocodeinone).

TABLE 14.2

RETENTION INDICES FOR SOME OPIUM ALKALOIDS AND CONGENERS ON SE-30¹²

	RI		RI
Codeine	2385	Methyldihydromorphine	2375
Cotarnine	1780	Morphine	2435
Desoxymorphine	2275	Nalorphine	2570
Heroin	2615	Noscapine	3100
Dihydrocodeine	2365	Oxycodone	2425
Dihydrocodeinone	2425	Oxymorphone	2520
Dihydromorphine	2440	Papaverine	2808
Ethylmorphine	2415	Pholcodine	2380
Dihydromorphinone	2490	Thebaine	2525

14.1.1.1.1. DERIVATIZATION

To obtain a better gas chromatographic identification of alkaloids containing phenolic or alcoholic hydroxyl groups, Anders and Mannering¹⁴ introduced on-column derivatization of the free base (*i.e.* morphine) with acetic anhydride and/or propionic anhydride. Morphine reacted completely with acetic anhydride giving two peaks, one of heroin and one, possibly, of 6-O-monoacetylmorphine, and with propionic anhydride to give only one peak, probably of di-propionylmorphine. The usefulness of the on-column derivatization technique depends upon the relationship between the retention times of the derivatives formed. The same technique was employed by Mulé¹⁵ for a number of opium alkaloids and related compounds, as will be seen from Table 14.3.

TABLE 14.3

RETENTION DATA OF SOME OPIUM ALKALOIDS AND CONGENERS¹⁵

Column: 6 ft by 3 mm I.D., SE-30 on Gas Chrom S (80-100 mesh) at 215°C

	Free*	Retention time (min)					
		With 5 μ l acetic anhydride		With 5 μ l propionic anhydride			
	t_R	t_R (rel)**					
Morphine	6.94	1.16	8.06	11.34	10.50		18.37
Normorphine	6.94	1.16	23.81	29.34	31.70	35.62	57.37
Codeine	6.0	1.00	6.0	8.40	6.0		10.54
Norcodeine	6.0	1.00	16.12	22.50	19.78		34.87
Heroin	11.34	1.89		11.34		11.34	
Nalorphine	9.84	1.64	11.62	15.94	14.81		26.34
Methyldihydromorphinone	7.50	1.25		10.12		12.56	
Dihydromorphinone	7.31	1.22	9.84	11.44	12.37		18.94
Ethylmorphine	6.71	1.12	6.71	9.28	6.71		11.68
Dihydro-14-hydroxy-morphinone	8.81	1.47	12.56	15.94	15.62		26.63
Dihydromorphine	6.94	1.16	8.06	10.31	10.31		16.31
Dihydrocodeine	6.94	1.16		6.94	6.94		10.87
Dihydro-14-hydroxycodone	8.62	1.44		8.62		8.62	
6-O-monoacetylmorphine	8.62	1.44		11.34		14.67	

* t_R for free untreated compound, ** t_R (rel) to free codeine

Brochmann-Hanssen and Baerheim Svendsen¹⁶ observed that phenolic alkaloids, such as morphine and apomorphine, were often difficult to gas chromatograph because of adsorptive effects. Morphine generally gave only about one-half of the peak height which one would expect on the

basis of the amount applied. Apomorphine with two phenolic hydroxyl groups also produced badly tailing peaks. It was therefore recommended that phenolic alkaloids should be converted to their trimethylsilyl ethers, which give symmetrical peaks without any tailing on the chromatogram that are well suited even for quantitative determinations. Hexamethyldisilazane was utilized as silylating reagent. Fish and Wilson¹⁷ used N,O-bis(trimethylsilyl)acetamide for the same purpose in studies on morphine in urine.

To be able to separate and identify picogram quantities of morphine and codeine after extraction from biological fluids, Kogan and Chedekel¹⁸ adopted the method of Dahlström and Paalzow¹⁹, using pentafluoropropionic anhydride and off-column reaction with an electron capture (⁶³Ni) detector; an OV-22 column was used. Kaneshina et al.²⁰ described the gas chromatographic detection of morphine and codeine in opium poppies as free bases, acetyl- and trifluoroacetyl- as well as trimethylsilyl derivatives.

For investigations on morphine, its metabolites and congeners (codeine, morphine-N-oxide, norcodeine, normorphine and pseudomorphine), Yeh²¹ applied gas chromatography of the trimethylsilyl-, acetyl and trifluoroacetyl derivatives (Table 14.4).

TABLE 14.4

RETENTION TIMES (MIN) OF MORPHINE AND ITS CONGENERS²¹

Compound	SE-30 3 % 3 ft		OV-17 3 % 3 ft		OV-17 3 % 6 ft		
	205°C	215°C	205°C	215°C	200°C	230°C	250°C
Trimethylsilyl							
-codeine	-	7	-	7.7	45.4	13.4	6.5
-morphine	-	6.0	-	6.7	34.5	10.5	5.7
-morphine N-oxide	-	6.0	-	6.7	34.5	10.5	5.7
-morphine N-methyl iodide*	-	4.0	-	4.7	-	12.0	5.8
		+ 6.2	-	+ 8.2	-	-	-
-norcodeine	-	6.3	-	10.3	-	19	8.0
-normorphine	-	8.5	-	8.3	41.3	12.5	6.5
-pseudomorphine**	-	-	-	-	-	-	-
Acetyl							
-codeine	-	6.0	-	13.5	-	-	-
-morphine	-	8.0	-	24	-	-	-
-morphine N-oxide	-	7.8	-	23.2	-	-	-
-morphine N-methyl iodide	-	8.0	-	19	-	-	-
-norcodeine	-	19.7	-	13.5	-	-	-
-normorphine	-	27.5	-	19 (250°C)	-	-	-
Trifluoroacetyl							
-codeine	5.2	3	7.7	4	-	-	-
-morphine	4.4	2.8	5.1	3.5	15	-	-
-morphine N-oxide	6.0	4.5	7.0	-	20	-	-
-norcodeine	7.5	-	13.0	-	-	-	-
-normorphine	7.1	-	8.7	-	-	-	-
-pseudomorphine***	53	-	-	-	-	-	-

SE-30 3 % on Varaport 100-120 mesh, OV-17 3 % on Gas Chrom Q 60-80 mesh, * Morphine N-methyl iodide gave two peaks which may arise from thermal decomposition, ** Trimethylsilyl-pseudomorphine did not emerge from the column at 290°C after 1 hr., *** The retention time for trifluoroacetyl-pseudomorphine was obtained at a column temperature of 290°C

On-column detivatization with trifluoroacetylimidazole and heptafluorobutrylimidazole for quantitative determination of morphine and codeine was used by Brugaard and Rasmussen²². Docosane was utilized as an internal standard. The relative standard deviation obtained from the reproducibility tests varied for morphine and codeine between 1.1 % and 2.8 %. The hepta-

fluoroacetyl derivatives gave better reproducibility than the corresponding trifluoroacetyl derivatives: 1.6 % for morphine and 1.1 % for codeine respectively, compared with 1.9 % and 2.8 % respectively. The authors found further that "clean chromatogram" without interfering peaks could be achieved by using freshly distilled reagents stored in tightly capped dark bottles.

In analysis of morphine and codeine in biological material often only micro amounts have to be determined. Christophersen and Rasmussen²³ developed an on-column derivatization procedure for such determinations by perfluoroacylation of both compounds in combination with electron capture detection. Heptafluorobutyrylimidazole was used as derivatization reagent. Excess of reagent must be removed prior to entering the electron capture detector, otherwise it will depress the standing current and detector response long enough to interfere with the quantitation of the peaks of interest. The removal of excess of reagent was obtained with a precolumn venting system. The derivatization reaction was found to be complete with the model substances used: codeine, ethylmorphine and morphine. The minimum detectable amount of codeine was found to be about 100 pg and that of morphine about 20 pg. A packed glass column with 3 % SE-30 on Supelcoport at 200°C was used.

A number of various derivatization reactions have been used, as shown in Table 14.25. The derivatization has been carried out as off-column or on-column derivatization, mostly in order to give morphine and related alkaloids with phenolic or alcoholic hydroxyl groups better gas chromatographic properties. Especially for quantitative determination of morphine in opium or biological material such derivatization was necessary. Street et al.³ found, however, that the problem of adsorption of a phenolic alkaloid, such as morphine, because of "active sites" on the solid support could be solved by deactivation of the solid support by treatment of it (diatomaceous earth) with benzoyl chloride in pyridine before coating it with the stationary phase.

14.1.1.1.2. COMBINATION OF GLC WITH OTHER ANALYTICAL TECHNIQUES

A combination of gas chromatography with other analytical techniques has often been used to ascertain the presence of opium alkaloids in biological fluids. Jain et al.²⁴ detected morphine and codeine in urine extracts by GLC and confirmed their presence by TLC. Sine et al.²⁵ screened urine samples for morphine by TLC and each specimen was then examined by gas chromatography. Mule²⁶ utilized GLC detection of drugs of abuse as an adjunct confirmatory test following routine TLC of urine extracts; Watanabe et al.²⁷ made use of GLC-MS for the detection of morphine and heroin in urine. A similar technique was employed by Weber and Ma²⁸ for a rapid identification of morphine and codeine in opium. Smith²⁹ developed a method for the identification of suspected opium samples based on computerized GC-MS of three of the main opium alkaloids, morphine, codeine and papaverine. The separation of the three alkaloids was obtained on a 3 % OV-101 column on Chromosorb W HP.

14.1.1.2. *Quantitative determination*14.1.1.2.1. *MORPHINE IN OPIUM*

The observation made by Brochmann-Hanssen and Baerheim Svendsen¹⁶ that morphine, when gas chromatographed as free base, was to some extent adsorbed on the chromatographic column, was confirmed by Schmerzler et al.³⁰ and by Martin and Swinehart³¹. Brochmann-Hanssen and Baerheim Svendsen³² used tetraphenylethylene or laudanosine as internal standards in their assay of morphine in opium, the tetraphenylethylene eluting prior to, and the laudanosine eluting after the morphine trimethylsilyl ether. The most crucial step in the procedure was the extraction of the total alkaloids and the separation of the phenolic and the non-phenolic alkaloids. This was achieved by means of a strongly acidic cation exchange resin (extraction and purification) and a strongly basic anion exchange resin (separation of phenolic and non-phenolic alkaloids). Using standard curves for morphine with both internal standards, the morphine content in a number of opium samples was analyzed and the gas chromatographic values compared with the results obtained, using a modified Mannich method (official in the Pharmacopoea Nordica, 1st Edition).

TABLE 14.5

MORPHINE CONTENT IN OPIUM SAMPLES (%)³²

Opium sample	Gas chromatography*		Other methods
	Value determined	Calc. to anhydr. base	
U.S.P.	11.4	-	10.5**
UN 2A	14.0	14.9	13.5***
UN 15	16.5	17.5	16.1****
UN 38G	18.5	19.7	17.0***
			20.3****
UN 137A	15.1	16.1	13.8***
UN 25A	18.3	19.5	18.1***
			19.1****
UN E529	13.8	14.6	13.8***
UN E531	11.0	11.7	12.0***
UN 265	12.8	13.6	13.1***
UN E612	15.5	16.3	15.5***
UN E627	12.4	13.1	11.0***
UN E631	12.2	13.2	12.0***

* Average values based on two or more determinations. ** Opium assay U.S.P. XVI.

*** Modified Mannich method, Pharmacopoea Nordica Ed.I; sample without drying.

**** United Nations Secretariat; calculated to anhydrous base.

Martin and Swinehart³¹ found that morphine base gave a constant value only after approximately 24 chromatographic analyses of 25 μ l of a 0.1 % solution of morphine and codeine, thus indicating that some adsorption took place on the column. After sufficient chromatograms, the adsorption sites became saturated with morphine and the sorption-desorption processes became equalised. For the quantitative determination of morphine and codeine in opium, the trimethylsilyl ethers of the alkaloids were chromatographed, using squalene as an internal standard. The values obtained for morphine in a number of authentic opium samples are given in Table 14.6, together with the morphine values obtained with the method of the Austrian Pharmacopoeia, 9th Edition.

Paris et al.³³ estimated the morphine content in opium poppies after silylation.

Notwithstanding the observations mentioned above concerning adsorption of morphine when

TABLE 14.6

PERCENTAGE ANHYDREOUS MORPHINE IN OPIUM³¹

determined by GLC as trimethylsilyl ether and with the method of the Austrian Pharmacopoeia, 9th Edition

Opium sample	GLC-TMS	Austrian Pharmacopoeia
UN M-1	9.9	9.34
UN M-2	11.5	12.45
UN M-3	10.0	11.23
UN M-4	11.0	12.26
UN M-5	5.9	5.6

gas chromatographed as free base, Nieminen³⁴ describes a method for the quantitative determination of morphine and the other main alkaloids of opium without derivatization on a 3.5 % SE-30 column. The results are seen in Table 14.7.

TABLE 14.7

CONTENT OF ALKALOIDS IN OPIUM, OPIUM EXTRACT AND OPIUM TINCTURE³⁴

determined without derivatization on a 3.5 % SE-30 column

Sample	Test	Morphine Ph. Nordica	Codeine Gas chromatography	Papaverine	Noscapine	Thebaine
Opium	1	11.8	0.82	1.17	4.37	
	2	11.2	0.84	1.20	4.80	
	3	11.0	0.75	1.24	4.36	
	4	11.6	0.86	1.16	4.50	
	5	11.0	0.80	1.09	4.36	0.83
	6	11.4	0.78	1.19	4.47	0.90
	Mean	11.3	0.81	1.18	4.48	0.87
	Std. dev.	0.33	0.04	0.05	0.17	
	Coeff. of var. %	2.9	4.9	4.4	3.8	
Opium		9.8				
Opium extract		19.9	21.0	1.72	1.43	4.5
Opium tincture		0.93	1.08	0.16	0.18	0.32

Bechtel³⁵ utilized a strong polar stationary phase (HI-EFF 8B) 0.75 % on Chromosorb G HP for the same purpose and succeeded in determining the main opium alkaloids in opium extracts without any derivatization. The relative standard deviation for the individual alkaloids lay between ± 0.6 % and ± 8.8 %, and for morphine at ± 2.3 %. An internal standard was used for the evaluation. On a packed column with a 1:1 mixture of OV-17 and SE-30 (3 % on Varaport 30 and 5 % on Chromosorb W ASW), Furmanec³⁶ determined codeine, morphine, thebaine, papaverine and noscapine simultaneously and quantitatively without derivatization and with a standard deviation for the individual alkaloids between 0.05 and 0.18 %.

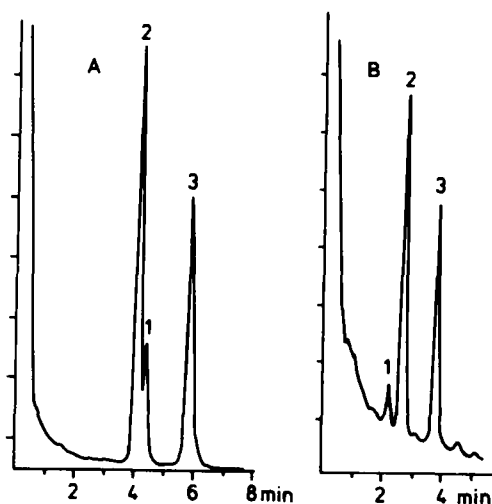
A simple and rapid method for gas chromatographic assay of the morphine and codeine content in opium was proposed by Nakamura and Noguchi³⁷ using N,O-bis(trimethylsilyl)acetamide both as a solvent for the opium sample to be analyzed and as derivatization reagent for morphine and codeine for the gas chromatographic analysis. Amount of 2-4 mg opium was introduced in a 3 ml glass-stopped tube, 200 μ l of 40 % N,O-bis(trimethylsilyl)acetamide was added and solution was effected by mechanical mixing on a Vortex vibrator. The 200 μ l of internal standard solution (2 mg nalorphine per ml pyridine) was added to the sample solution and mixed. One μ l of the resulting solution was injected for quantitative gas chromatographic analysis

on a 3 % OV-17 column and a 3.8 % UCW-98 column. Typical chromatograms are given in Figure 14.1.

FIGURE 14.1

CHROMATOGRAMS OF OPIUM EXTRACT³⁷

A: 3 % OV-17 column, B: 3.8 % UCW-98 column. 1 = codeine, 2 = morphine, 3 = nalorphine (internal standard) as trimethylsilyl derivatives.



A comparison of opium assay values is given in Table 14.8.

TABLE 14.8

COMPARISON OF OPIUM ASSAY VALUES³⁷

Method	Percent morphine	
	Opium UN-38 G	Opium UN-25 A
Present GLC method	17.92	18.92
GLC method of Brochmann-Hanssen and Baerheim Svendsen 1963	-	17.83
Isotope dilution method of Brochmann-Hanssen 1972	17.50	18.4-18.5
TLC method of Mary and Brochmann-Hanssen 1963	17.51	17.85
Mannich method (Baerheim Svendsen 1959)	17.00	18.10

In a report published by the Joint Committee of the Pharmaceutical Society and the Analytical Division of the Chemical Society on Pharmaceutical Analysis³⁸ a gas chromatographic method was proposed for the quantitative determination of morphine in opium and opiate preparations to replace the assay laid down in the British Pharmacopoeia. Several stationary phases were tried and a mixture of 4:6 of OV-25 and OV-225 was preferred. Since no complete recovery of morphine from the gas chromatographic column could not be obtained, when chromatographed as free base, silylation of morphine was recommended. Nalorphine was used as an internal standard, as proposed by Wallace et al.⁵¹, since it is structurally identical to

morphine in the vicinity of the hydroxyl group and reacts similarly to morphine by the silylation reaction. The values obtained in eight laboratories for the morphine and codeine content in five United Nations raw opium samples are listed in Table 14.9.

TABLE 14.9

MORPHINE AND CODEINE CONTENT IN UNITED NATIONS RAW OPIUM SAMPLES³⁸

Column: 1.5 m by 4.0 mm I.D., glass, 1 % of a 4:6 mixture of OV-225 and OV-25 on Diatomite CQ or Gas Chrom Q (100-120 mesh) at 200-210°C.

Laboratory	UN-M-1		UN-M-2		UN-M-3		UN-M-4		UN-M-5	
	Morph.%	Cod.%	Morph.%	Cod.%	Morph.%	Cod.%	Morph.%	Cod.%	Morph.%	Cod.%
1	11.57	4.34							7.04	3.16
	11.59	4.25							7.19	3.23
2	11.36	3.73	14.23	2.24	13.82	1.28	12.98	3.51	6.93	2.86
	11.27	4.26	14.63	2.60	14.22	1.35	13.05	3.50	7.09	2.97
3	11.21	3.92	15.12	2.32	13.65	1.20	13.34	3.63	7.04	2.65
	11.29	4.04	14.74	2.30	13.47	1.19	13.32	3.62	7.09	2.83
4	11.0	4.10	14.50	2.36	14.39	1.36	13.17	3.54	7.16	2.89
	11.45	3.99	14.11	2.36	14.20	1.34	13.46	3.43	7.23	3.20
5	11.69	4.29	14.30	2.55	13.86	1.41	12.76	3.56	6.89	2.83
	11.50	4.27	14.46	2.51	13.85	1.42	12.64	3.52	6.91	2.73
6	12.01	3.49	14.75	2.56	14.05	1.45	13.28	3.55	7.23	2.92
	11.80	3.45	14.71	2.59	14.00	1.47	13.10	3.54	7.21	2.86
7	10.17	3.80	15.64	2.54	14.01	1.37	12.64	4.11	6.58	2.77
	10.42	3.75			14.07	1.35	12.53	4.03	6.36	2.68
8	11.72	3.83								
	11.84	4.02								
Mean	11.37	3.97	14.65	2.45	13.97	1.35	13.02	3.62	7.00	2.90
Stand.dev.	0.49	0.28	0.43	0.13	0.25	0.09	0.31	0.21	0.25	0.18
Coeff.var. %	4.35	7.07	2.95	5.4	1.82	6.57	2.41	5.88	3.63	6.36

Two aliquots from a single weighing of each opium sample were chromatographed by each laboratory.

An elegant and rapid method for quantitative determination of morphine in opium extracts was published by Rasmussen³⁹ using trimethylsilylimidazole for on-column silylation of morphine and *n*-tetracosane (C₂₄) as an internal standard. The gas chromatography was carried out on a 3 % Dexsil 300 column. Analysis of a standard opium extract with 14.9 mg/ml of morphine gave an average content of 15.0 mg/ml and a coefficient of variation of 1.4 %.

14.1.1.2.2. MORPHINE IN BIOLOGICAL MATERIALS

For the determination of morphine in opium, Ikekawa et al.⁴⁰ developed a rapid method whereby morphine and morphine glucuronide were isolated from the urine sample (50 ml) by adsorption on a charcoal column (1 g in a column of 1 cm I.D.), washed with water to remove impurities, and eluted with glacial acetic acid (20 ml). After evaporation to dryness in vacuo, the morphine glucuronide was hydrolyzed with hydrochloric acid, and the pH adjusted to 2.5 with sodium hydroxide and extracted with chloroform:isopropanol (9:1). The water layer was

adjusted to pH 9.0 and extracted with the same solvent. The dry residue of the solution obtained was dissolved in N,O-bis(trimethylsilyl)acetamide (0.1-0.2 ml) and after 10 minutes at room temperature 10 μ l was injected for gas chromatographic analysis. Nalorphine was used as an internal standard - and was added to the urine sample before any treatment. The overall recovery was about 60 %. 5-10 μ g morphine in 1000 ml urine was the limit of detectability with this method. Packed columns (1.8 m by 4 mm I.D.) with 1.5 % OV-1, 1.5 % OV-17, 1.5 % SE-30, 1.5 % QF-1 or 1.5 % XE-60 on Shimalite W 80-100 mesh were used for the gas chromatography.

Payte et al.⁴¹ stated that the sensitivity of detection of morphine in urine could be increased by acid hydrolysis of the glucuronide metabolite. Later Yeh et al.⁴² showed that 50 % of the morphine found in urine was as morphine,3-glucuronide.

Truhaut et al.⁴³ investigated the detection of free morphine in the urine of drug addicts and used a mixture of chloroform:ethyl acetate:ethanol (3:1:1) for the extraction, after adjustment of the pH to 8-8.5 with bicarbonate. Morphine was derivatized with N,O-bis(trimethylsilyl)acetamide and the gas chromatography was carried out on a 3 % Dexsil column using tetracosane (C₂₄) as an internal standard. 5 μ g in 10 ml urine could be determined by this method.

A rapid and simple method for total morphine in urine was developed by Fry et al.⁴⁴ using ¹⁴C morphine to correct for extractive losses. Morphine conjugates were hydrolyzed by β -glucuronidase and morphine extracted with ether. Nalorphine was used as an internal standard and both compounds (morphine and nalorphine) were converted into their trimethylsilyl ethers using N,O-bis(trimethylsilyl)acetamide as reagent. By adding ¹⁴C morphine to the urine sample to be extracted and analyzed no attempt was made to extract the morphine quantitatively, since the final result could be corrected for recovery by the use of ¹⁴C-morphine. The coefficient of variation over a period of six months was 5.6 %. A 90 cm long packed column with Chromosorb G coated with a mixture of 0.35 % JXR and 0.35 % CDMS was used at 210°C.

The mass fragmentographic technique with stable isotopes as internal standards shows high sensitivity (in the picogram range), specificity due to the focusing on specific mass fragments, and good reproducibility in quantitative work because the internal stable isotope standard makes it possible to correct for losses in the preparative phase of the analysis. Ebbighausen et al.⁴⁵ developed a method for the assay of morphine and codeine in urine using deuterium labeled morphine and codeine. Codeine was derivatized with heptafluorobutyric acid and morphine with trifluoroacetic acid before gas chromatography on a packed column with 1 % OV-17 on Gas Chrom Q. The LKB 9000 combination of gas chromatograph-mass spectrometer equipped with a multiple-ion detector/peak matcher was used. With a simple extraction procedure of 1 ml urine after hydrolysis a practical limit of detectability was found to be approximately 500 pg.

Clarke and Foltz⁴⁶ developed a similar method for the analysis of morphine in urine with N-C²H₃-morphine as internal standard. The internal standard was added to 10 ml urine, the urine buffered to pH 8.5 and extracted with chloroform:isopropanol (4:1). The extraction residue was trimethylsilylated by adding 25 μ l of N,O-bis(trimethylsilyl)acetamide and heating at 60°C for about 1 h. About 2 μ l was analyzed on a 3 % OV-17 column at 230°C coupled direct to a Finnegan 1015 quadrupole mass spectrometer equipped with a chemical ionization source, which was operated at an ionizing energy of 100 eV, an ion repeller voltage of 0 V and a filament emission of 300 μ A. The mass spectrometer was interfaced with a System Indus-

tries 250 computer system. Morphine concentrations as low as 5 ng/ml could be measured by selecting ion monitoring.

Wilkinson and Way⁴⁷ developed a method for quantitative determination of morphine in plasma and cerebrospinal fluid (0.1-1.0 ml). Morphine was extracted with ethyl acetate containing 10 % isopropanol, back extracted with hydrochloric acid and the residue of this solution treated with N,O-bis(trimethylsilyl)acetamide containing 1 % trimethylchlorosilane (25 μ l). The amount of morphine was determined using tetraphenylethylene as internal standard on a packed column with 3 % OV-10 as stationary phase at 215°C. Limit of sensitivity of the method was found to be about 25 ng per sample.

To be able to determine morphine at sub-microgram levels in urine, bile, whole blood and tissue homogenates with a high degree of specificity, Wallace et al.⁴⁸ converted morphine to its diacetyl derivative prior to gas chromatography. Since morphine is excreted as the glucuronide conjugate, acid hydrolysis of the urine sample was recommended to enhance the sensitivity of detection. The recovery of morphine after in vitro addition to urine and homogenized liver is summarized in Table 14.10.

TABLE 14.10

RECOVERY OF MORPHINE AFTER IN VITRO ADDITION TO URINE AND HOMOGENIZED LIVER⁴⁸

Amount added μ g/ml	Recovery, mean \pm standard deviation (μ g/ml)	
	Urine	Liver
2.5	2.44 \pm 0.03	2.0 \pm 0.12
5.0	4.75 \pm 0.13	3.76 \pm 0.13
10.0	9.36 \pm 0.33	7.40 \pm 0.20
50.0	47.02 \pm 2.05	(not determined)
Average recovery	95.1 %	76.4 %

The average recovery for morphine as determined by the gas chromatographic technique and calculated from the mean value for each concentration.

Garrett and Gürkan⁴⁹ compared a number of sensitive methods for the determination of morphine in biological fluids and found no significant differences among: a method based on liquid scintillation, a radioisotope method and electron capture gas chromatography. The morphine was determined by GLC at 215°C on a 3 m long column as the pentafluoropropionate derivative with nalorphine pentafluoropropionate as an internal standard. The estimated standard deviation (in percent of concentration) of an assay from 0.5 ml of plasma ranged from 1 % at 2500 ng/ml to 9.2 % at 5 ng/ml.

To be able to measure morphine levels in brain in connection with studies on the concentration of morphine and analgetic activity, Hipps et al.⁵⁰ utilized the multiple ion detection method (mass fragmentography) using a computer-controlled gas chromatograph-mass spectrometer (DP-12 - LKB-9000 GC-MS). The morphine was converted to its trifluoroacetyl derivative immediately before the analysis and the gas chromatography was carried out on a 50 cm long by 6 mm O.D. glass column packed with 3 % SE-30 on Gas Chrom Q at 210°C. Deuterium labeled trifluoroacetylmorphine (N-methyl-²H₃-morphine) was used as an internal standard. The authors stressed that the power of the multiple ion detection method is partly due to the sensitivity attainable using the mass spectrometer as a gas chromatographic detector, but, more significantly, to the fact that fragment ions in the mass spectra of substances can be used as chemically selective detectors.

Investigators have derivatized morphine with a variety of reagents, achieving both superior gas chromatographic characteristics and enhanced sensitivity. In many cases pre-chromatographic derivatization of morphine may be advantageous, utilizing reagents such as trifluoroacetic anhydride to give trifluoroacetyl morphine, which can be detected in a quantity of less than 0.1 nanogram, when using a cylindrical rod ^{63}Ni electron capture detector⁵¹. Since the derivatization is quantitative and the gas chromatograph response is linear, with the concentration over a wide range - also for nalorphine, used as an internal standard - quantitative determinations of morphine can be carried out in biological fluids. The method of Wallace et al.⁵¹ offers possibilities of determining plasma or serum levels down to 25 ng/ml of morphine in a 2 ml specimen with acceptable precision, although concentrations of 5 ng/ml are detected by this procedure. Nalorphine, structurally identical to morphine in the vicinity of the hydroxyl group, reacts similarly to morphine and thus serves as a control both for the derivatization reaction and for variations in the chromatographic technology.

Nakamura and Way⁵² determined morphine and codeine in cadaverous body fluids and tissue samples after extraction with organic solvent and derivatization with N,O-bis(trimethylsilyl)acetamide, using nalorphine as an internal standard and OV-17 3 % and UCW-98 3.8 % as stationary phases.

Because of the higher stability of pentafluoropropionyl morphine compared with trifluoroacetyl morphine and its higher electron capture properties, Dahlström and Paalzow¹⁹ preferred this derivative for a method for quantitative determination of morphine in biological samples, using electron capture (tritium foil) detector. Nalorphine was used as an internal standard. The sensitivity limit of the detector was about 5 pg of morphine and the corresponding limit when extracted from plasma about 500 pg ml⁻¹. In the brain, 100 pg morphine could be determined in a 30 mg brain tissue sample. Analyses of nine different plasma samples containing 0.750 ng ml⁻¹ morphine, showed a variation coefficient of 5.3 ± 1.2 %. The sensitivity is thus in the same range as reported for the immunological technique. Furthermore, gas chromatography has the advantage of combining rapidity and specificity with high sensitivity and selectivity of the electron capture detector.

Derivatization of codeine and morphine with pentafluoropropionic anhydride using electron capture detection, as described by Dahlström and Paalzow¹⁹ was used by Dahlström et al.⁵³ for simultaneous determination of codeine and morphine in plasma and brain samples. The alkaloids were extracted from 0.05-1.0 ml samples after addition of the internal standards (3-ethylmorphine and N-ethyl-normorphine) and carbonate buffer to adjust the pH to 8.9, with toluene:butanol (9:1). Back extraction with acid and re-extraction with toluene:butanol gave a dry residue of the alkaloids, which was treated with pentafluoropropionic anhydride for 30 min at 65°C. Excess of reagent was removed and the sample dissolved in ethyl acetate for the gas chromatographic analysis on a 3 % OV-17 column on Gas Chrom Q 100-120 mesh at 215°C. The sensitivity of the method (0.75 ng of morphine and 7.5 ng of codeine in a sample) makes it useful for pharmacokinetic investigations. The reproducibility was good.

Pentafluorobenzoylation of morphine and related phenolic alkaloids by extractive alkylation was used by Cole et al.⁵⁴ for the determination of morphine in plasma by gas chromatography and mass fragmentography using morphine-d₃ as internal standard. The extractive alkylation affords a method of isolating polar compounds such as morphine with simultaneous derivatization. To 1 ml of plasma containing unknown amounts of morphine, the internal standard was added, as well as trifluoroacetic anhydride, pentafluorobenzyl bromide, alkali and ethyl acet-

ate as solvent to remove the reaction products after their formation. By back extraction, first with acid and water, then with ethyl acetate after basification, the GC-MS was carried out using a packed column with 3 % OV-17 on Diatomite C at 265°C in an MS 30 gas chromatograph mass spectrometer (AEI). When using pentafluorobenzyl bromide derivatives no impurities with long gas chromatographic retention times were encountered, such as when using the di-trifluoroacetic anhydride derivatives.

Anders and Mannering¹⁴ introduced on-column derivatization of morphine with propionic anhydride and obtained only one peak. They believed that it was dipropionylmorphine. Von Meyer et al.⁵⁵ made use of the same derivatization reaction for the quantitative determination of morphine in blood, but they found that the monopropionyl derivatives of morphine and nalorphine (used as an internal standard) were found in the proportion 99:1, as compared with the dipropionyl derivatives. The extraction of blood was performed by means of Extrelut^R with petroleum ether 40°C:isoamylalcohol (1:1) and the gas chromatography was carried out on a 3 % OV-1 on Chromosorb W HP packed column at 230°C. Good results were obtained in the range of 300-600 ng/ml blood.

Cimbura and Koves⁵⁶ applied an other extraction procedure for morphine from blood samples: adsorption on an XAD-2 resin and subsequent elution with an organic solvent mixture. Morphine was derivatized with acetic anhydride prior to gas chromatography, which was carried out on a 3 % OV-17 on Chromosorb W HP packed column at 240°C. An overall recovery of 70-80 % was achieved and an overall precision of the method with a coefficient of variation of 4.2 %. The detection limit was 0.05 mg/l using a NP-detector.

Saady et al.⁵⁷ preferred acylation of morphine with N-methyl-bis-trifluoroacetamide for its determination in blood and serum. Extraction was performed at pH 9.9 with toluene:hexane:isoamylalcohol (78:20:2), and the extraction efficiency of the method was found to be between 55 and 60 %. The use of nalorphine as an internal standard, coupled with the sensitivity of the GC-MS, did not necessitate further extraction. Quantitative determinations were carried out from less than 0.2 mg/l to at least 8 mg/l for morphine and codeine with good results.

The problem of adsorption of a phenolic alkaloid such as morphine during gas chromatography because of "active sites" on the solid support (diatomaceous earth) has mostly been avoided by converting morphine to derivatives such as acetyl, propionyl, fluoroacetyl, fluoro-propionyl and silyl derivatives. Street et al.³, however, developed a technique for preparation of low activity packed columns for gas chromatography of polar compounds, i.e. morphine, in unmodified form in nanogram amounts using a nitrogen-specific detector.

The diatomaceous earth (Chromosorb G) was washed with acid and water, dried and acylated by treatment with benzoyl chloride in pyridine 48 h. After washing with acetone and drying, the support was coated with SE-52 and dried under nitrogen in a Pyrex tube (I.D. 4 cm) and then heated at 410°C - also under nitrogen. Pyrex columns (6 ft by 3 mm O.D.) were cleaned with concentrated hydrochloric acid, washed with water and acetone and dried. Then they were filled with a mixture of benzoyl chloride and pyridine (2:3) and kept for three days, emptied, rinsed with toluene and dried at 100°C.

Two variations of deactivation were performed;

(A) A 5 % solution of SE-52 in methylene chloride was drawn by suction into three windings of the column and slowly moved through it to the other end. This procedure was repeated and, after pouring out the excess liquid, the column was dried first at 100°C and finally at 350°C under nitrogen for 10 h. The glass column was then ready for filling.

(B) The glass column was filled with coated support material and heated at 400°C under nitrogen for 1 h, then this powder was poured out. The column was refilled with coated support and heated under nitrogen at 350°C in the gas chromatograph until a stable baseline was obtained.

The gas chromatography was carried out at a column temperature of 250°C for morphine base. When decreasing amounts of morphine were chromatographed on a commercial OV-1 column and on the proposed deactivated column, and graphs showing the relationship between peak height and amounts of morphine were constructed, it was found that the commercial OV-1 column had a cut-off point at 0.4 µg morphine, whereas the proposed deactivated column had a cut-off point at 0.8 ng. Hence, the proposed column showed an improvement in detection limit of the order of 1000-fold.

Coating of the glass surface as well as the diatomaceous earth (Procedure A) produced results for morphine that showed less adsorption relative to a C₂₄ hydrocarbon than when diatomaceous earth only was treated. With the nitrogen-specific detector there was, however, only a slight difference between the methods A and B over a range of about 25-100 ng of morphine.

The proposed column showed good thermal stability over at least 18 months under routine conditions of a busy toxicology laboratory.

14.1.1.2.3. NON-PHENOLIC ALKALOIDS

The principal non-phenolic alkaloids were determined by Baerheim Svendsen and Brochmann-Hanssen⁵⁸ on a SE-30 column, 4 %. Although the solid support, Gas Chrom P, was acid and base washed, silanized and precoated with 0.1 % of polyethylene glycol 9000 before coating with SE-30, appreciable amounts of the alkaloids were adsorbed on the column, the adsorption increasing in magnitude with an increase in the retention time of the alkaloid. Although the chromatographic peaks showed satisfactory symmetry, this is in itself no guarantee against loss of sample through adsorption. For any one alkaloid the amount adsorbed appeared to be fairly constant and reasonable independent of the sample size. Consequently, the percentage of the injected sample lost by adsorption decreased as the sample size increased. Thus it was not possible to obtain reproducible values for the correction factors. The same difficulties were experienced with the unknown solution to be analyzed. Under such circumstances a quantitative determination was subject to errors of considerable magnitude. Nevertheless, a selection of authentic opium samples were analyzed, using the average correction factors, given in Table 14.11. The analytical results are given in Table 14.12.

By means of ion-pair extraction with diethylhexyl phosphate and gas chromatography on an OV-17 column Guttman et al.⁵⁹ determined papaverine in plasma at levels as low as 0.01 µg/ml. To reduce any potential adsorption of papaverine by the gas chromatographic column, three injections of a concentrated solution of papaverine and the internal standard, dibucaine, in diethyl ether were made prior to any series of analyses.

Nieminen³⁴ and Bechtel³⁵ do not mention any loss of alkaloids due to adsorption on the chromatographic column of the principal non-phenolic alkaloids in opium. They used respectively SE-30 (3.5 %) and HI-EFF 8B (0.75 %) as stationary phases.

Quantitative determinations of codeine in pharmaceutical preparations, e.g. in mixtures with other compounds, were carried out by Wesselman⁶⁰ on silicone gum (Linde W-98), by Stev-

ens⁶¹ on OV-17 and by Dechene et al.⁶² on Dow Corning Fluid No.200. Nieminen⁶³ determined codeine phosphate in pharmaceutical preparations after extraction of the free base, utilizing a UCC-W 982 10 % column.

TABLE 14.11

CALCULATION OF CORRECTION FACTORS BASED ON GLC OF ALKALOID MIXTURES OF KNOWN CONCENTRATIONS⁵⁸

(1) Concentration of alkaloid in solution (mg/ml), (2) Peak area (arbitrary units), (3) Correction factor based on strychnine, (4) Average value of correction factor.

	Codeine			Thebaine			Papaverine			Strychnine			Noscapine		
	a	b	c	a	b	c	a	b	c	a	b	c	a	b	c
(1)	1.5	2.25	6.0	1.0	2.25	4.0	3.5	2.5	1.5	5.0	5.0	5.0	6.75	7.5	7.5
(2)	117	83	444	103	128	372	215	73	73	175	88	150	235	147	230
(3)	0.45	0.43	0.40	0.34	0.28	0.30	0.57	0.60	0.62	1.00	1.00	1.00	1.01	0.90	0.92
(4)	0.427			0.307			0.597			1.00			0.963		

TABLE 14.12

GLC DETERMINATION OF CODEINE, THEBAINE, PAPAVERINE AND NOSCAPINE IN OPIUM⁵⁸

The values in parentheses obtained with other methods.

Sample	Codeine %		Thebaine %		Papaverine %		Noscapine %	
UN 2A	1.97	(2.07)	1.22	(2.08)	1.03	(1.44)	6.52	(6.67)
UN 15	1.21	(1.33)	1.38	(1.75)	1.80	(1.80)	5.80	(6.18)
UN 25A	1.38	(1.50)	0.66	(1.15)	2.18	(3.34)	6.69	(6.66)
UN 27A	2.45	(2.35)	3.02	(3.21)	1.00	(1.35)	6.19	(6.28)
UN 37C	5.82	(4.07)	2.73	(3.23)	1.13	(1.40)	7.05	(6.68)
UN 38G	1.76	(1.52)	1.15	(1.28)	2.57	(3.19)	6.29	(6.47)

Wesselman and Koch⁶⁴ determined codeine phosphate together with metapyrilene fumarate and ephedrine hydrochloride in syrup after extraction with chloroform of the basified syrup and addition of the internal standard, amobarbital. The gas chromatography was carried out by temperature programming from 145 to 225°C at a heating rate of 10°C/min on a 3.8 % Linde W-98 silicone gum on Diatoport S column. The relative standard deviation for the three components were ± 3.76 % (ephedrine), ± 2.38 % (metapyrilene) and ± 2.02 % (codeine).

Schmerzler et al.³⁰ found that codeine and its metabolite norcodeine could not be separated on a SE-30 column, and peaks observed represented any contribution from either compound. However, under mild acetylation conditions there is a great difference in reaction rate between codeine and norcodeine. This was used to remove norcodeine before injection on SE-30 columns. Norcodeine may be estimated by difference if an injection before acetylation is made first. However, on an XE-60 column the two alkaloids were resolved as free bases and acetylation was unnecessary. On an OV-17 column Serfontein et al.⁶⁵ determined codeine extracted from serum by microphase extraction, whereas Brunson and Nash⁶⁶ made measurements of codeine and norcodeine in plasma, whereby norcodeine was derivatized with ethyl chloroformate.

For the gas chromatographic determination of codeine in plasma Zweidinger et al.⁶⁷ utilized *n*-butyl chloride for the extraction of codeine from plasma instead of chloroform, which has been mostly used, because it does not readily give emulsions with plasma such as chloroform. On an OV-17 column or an XE-60 column quantitative determinations in low nanogram quantities (50 ng/ml) were carried out, but the limit of detection was as low as 5 ng/ml. Dihydrocodein-

one was used as an internal standard.

In a paper on a comprehensive gas chromatography procedure for measurement of drugs in biological material Shipe and Savory⁶⁸ also studied morphine and codeine. The drugs were extracted from 1 ml of serum or urine after addition of buffer, and gas chromatographed on a 3 % OV-17 column by temperature programming.

There has been an increased interest in *Papaver bracteatum* Lindl. because of the relatively high content of thebaine in its latex, capsules, leaves and roots, and a number of analytical methods for its quantitative determination have been developed, among them gas chromatographic methods. The extraction of the alkaloid from the plant material and its purification without losses prior to gas chromatography, seems to be a major problem. Ping Chen and Dorenbos⁶⁹ and Vincent et al.⁷⁰ used extraction with acetic acid (3-5 %), removal of i.a. pigments with diethyl ether or hexane, and isolation of the alkaloid after adjustment of the pH to 9-9.5 by extraction with diethyl ether or chloroform - followed by gas chromatography. The gas chromatography has mostly been done on an OV-17 packed column^{69,71,72,73,74,75,76,77,78}. The method developed by Fairbairn and Helliwell⁷³ gave a satisfactory reproducibility with a wide range of thebaine content (0.1-36 %) in latex, capsule, roots and leaves. The coefficient of variation for single assays ranged from 0.63 to 3.3 %. The accuracy was almost 100 % with recovery experiments and examination of the marks left after the assay process. No decomposition of thebaine seemed to take place, since an almost 100 % recovery was obtained using added thebaine or thebaine alone.

Fen-Fen Wu and Dobberstein⁷⁵ found that the compound eluted from the gas chromatographic column and recorded as a thebaine peak on the chromatogram, did not contain any thebaine as analyzed by TLC, if a column temperature of 270°C was used in the gas chromatography. However, when gas chromatographed at 225°C, a single thebaine spot was observed with TLC. At this low temperature severe tailing of the thebaine peak was observed, making accurate quantification difficult.

Küppers et al.⁷¹ stated that more reproducible quantitative results were obtained when thebaine base was gas chromatographed, than with thebaine hydrochloride. Vincent et al.⁷⁰ observed that a small amount of thebaine was always adsorbed during GLC (< 1 %)(new OV-17 2 % on Gas Chrom Q columns). The columns were therefore saturated with an alkaloid standard containing 0.2-0.4 µg thebaine by injection. By carefully standardizing and checking each step of the procedure for loss of thebaine, the method was assumed to be suitable for intra- and inter-laboratory analyses.

For most gas chromatographic analyses cholesterol acetate was utilized as an internal standard.

For the determination of papaverine in the blood of rats and dogs, Mussini and Marzo⁷⁹ developed a method based on the extraction of the alkaloid with diethyl ether from 2 ml blood, after basification with sodium hydroxide, back extraction with hydrochloric acid and extraction with diethyl ether (after adjustment of the pH to 10). To the dried residue a solution of the internal standard (penfluridol) was added and the gas chromatographic analysis carried out on a packed column with 3 % OV-17 on Gas Chrom Q at 290°C, and a ⁶³Ni electron-capture detector. The sensitivity of the method was 10 ng/ml blood.

De Graeve et al.⁸⁰ described two methods for the papaverine determination in blood samples for pharmacokinetic studies, one utilizing a packed column with OV-1 1 % and interfaced with a LKB 9000 S mass spectrometer equipped with a multiple ion detector for mass fragmentography,

and the other using a capillary column with SE-30 as stationary phase and flame ionization detection. Owing to its high specificity, the mass fragmentographic method was greatly superior to capillary gas chromatography, which was sometimes subject to interference by solvent impurities. Under selected conditions, a precision of about 2 % was obtained by the mass fragmentography technique, the reproducibility of the overall method was 10 % and the limit of detection in blood about 5 ng/ml.

To omit unconventional instrumentation that was not suitable for routine analysis, as proposed by De Graeve et al.⁸⁰, Bellia et al.⁸¹ developed a quite simple method to determine papaverine in blood samples, using conventional flame ionization or a nitrogen-phosphorus detector. The internal standard, strychnine, was added to the sample prior to extraction, which was carried out with toluene after basification, back extraction with acetic acid and extraction of the liberated base with diethyl ether. The gas chromatography was done on a packed column with 2 % OV-101 at 275°C. To minimize the adsorption effects, the column was silanized by *in situ* injection and by injection of a concentrated solution of papaverine and the internal standard prior to routine analysis. Precision and accuracy of the method is shown in Table 14.13.

TABLE 14.13

PRECISION AND ACCURACY OF PAPAVERINE ANALYSIS IN BLOOD⁸¹

Each result is the mean of four determinations. SD = standard deviation, RSD = relative SD

Nanogram added	Nanogram found	SD	RSD (%)	Detector
20.0	19.8	2.5	12.7	NPD
40.0	40.0	2.7	6.7	NPD
80.0	79.5	3.9	4.9	NPD
160.0	159.5	4.0	2.6	NPD
100.0	100.3	10.5	10.5	FID
250.0	250.0	26.9	10.8	FID
500.0	502.5	20.6	4.1	FID
1000.0	995.5	56.9	5.7	FID

Gazdag and Nyiredy⁸² applied gas chromatography to determine ethylmorphine hydrochloride (20 mg) in tablets also containing phenacetin (300 mg) and aminophenazone (300 mg). On a SE-30 packed column the compounds were separated and could be determined, the ethylmorphine hydrochloride had a relative standard deviation of ± 2.7 %.

In investigations on various drugs of abuse in human biological fluids (urine and plasma) and tissues, there has also been a need for the determination of narcotic antagonists. Digregorio and O'Brien⁸³ made use of gas chromatography on an OV-17 3 % column for such investigations after extraction of the compounds from alkalinized urine with chloroform and conversion into the corresponding trimethylsilyl ethers. The recoveries of each compound varied from 62 to 78 % for naloxone, depending on concentration, to 97 to 100 % for naltrexone.

Smith and Stocklinski^{84,85} studied the metabolites of apomorphine (apocodeine, isoapocodeine, norapocodeine) in urine and faeces of rats, and worked out a method for the determination of apomorphine as trimethylsilyl ether and the metabolites as free bases on an OV-17 3 % column. Average recovery of apocodeine and isoapocodeine was between 85 and 90 % for 500-1000 μ g.

Because of the lack of selectivity of this method, Baaske et al.⁸⁶ developed a method

using derivatization of apomorphine with heptafluorobutyric anhydride. N-n-propyl-norapomorphine was utilized as an internal standard. Amounts of 1 to 10 µg apomorphine per ml plasma could be determined. Quantitative relative recoveries with a relative standard deviation of 4.6 % were achieved by the method, which also permits analysis of apomorphine in the presence of its two monomethyl ether derivatives, apocodeine and isopapocodeine. The latter compounds were chromatographically resolved as their heptafluorobutyrate derivatives.

14.1.1.2.4. HEROIN

Brochmann-Hanssen and Baerheim Svendsen¹⁶ found that heroin was eluted as a sharp peak when gas chromatographed alone on a SE-30 column. However, in mixtures with codeine, morphine or other alcoholic or phenolic substances, reactions taking place in the injector gave rise to several new esters, not present in the original solution. Both 3-O-acetyl- and 6-O-acetylmorphine gave single peak chromatograms in the absence of glass wool in the column entrance. An apparent catalytic effect of glass wool resulted in peaks corresponding to morphine, monoacetylmorphine and heroin. Curry and Patterson⁸⁷ combined infrared spectroscopy, thin-layer and gas-liquid chromatography for the analysis of illicit heroin samples, whereby commonly found alkaloids, such as morphine and the monoacetylmorphines were taken into account. However, no problems concerning the gas chromatography of heroin in mixtures with morphine, as mentioned by Brochmann-Hanssen and Baerheim Svendsen¹⁶ were mentioned. A packed column with cyclohexane succinate 3 % and dibenzyl phthalate as an internal standard were used.

For quantitative determination of heroin in illicit samples in the presence of alkaloids such as morphine and monoacetylmorphine Grooms⁸⁸ silylized the sample prior to the gas chromatography, utilizing cinchonine as an internal standard. De Zan and Fasanello⁸⁹ determined heroin hydrochloride in illicit preparations by directly injecting a sample solution containing an internal standard (cholesterol) without prior extraction, whereby the most common adulterant in heroin street dosage preparations, quinine hydrochloride, could be determined on an 3 % OV-1 column. The same stationary phase was employed by Moore and Bena⁹⁰ for the analysis of heroin in narcotic paraphernalia.

Shaler and Jerpe⁹¹ combined gas chromatography and infrared spectroscopy for the identification of heroin and commonly used diluents, such as quinine and procaine in illicit seizures. The quantitative determination of heroin was carried out gas chromatographically using cholesterol TMS as an internal standard.

To be able to compare heroin samples sold in illicit channels through the determination of impurities present, such as 6-O-monoacetylmorphine, acetylcodeine, morphine and codeine, Sobol and Sperling⁹² developed a method for quantitative determination of heroin and the compounds mentioned. Two chromatograms were run of each sample: one after derivatization with N,O-bis(trimethylsilyl)trifluoroacetamide and one by direct gas chromatography of the sample - in both cases on a packed column with 3 % OV-25 on Gas Chrom Q. Typical chromatograms are given in Figure 14.2.

The analyses were limited to specific exhibits where intelligence had indicated a probable connection between two or more cases and the laboratory examination was carried out to prove or disprove this connection. A constant relationship of the relative concentrations of the by-products were found in samples from a common source (No. 1771-1832 in Table 14.14) whereas the spectrum differed in a sample from another source (No. 1833 in Table 14.14).

FIGURE 14.2

GAS CHROMATOGRAMS OF HEROIN SAMPLE⁹²

Column: OV-25 3 % on Gas Chrom Q, Derivatized heroin at 240°C, Underivatized heroin at 265°C

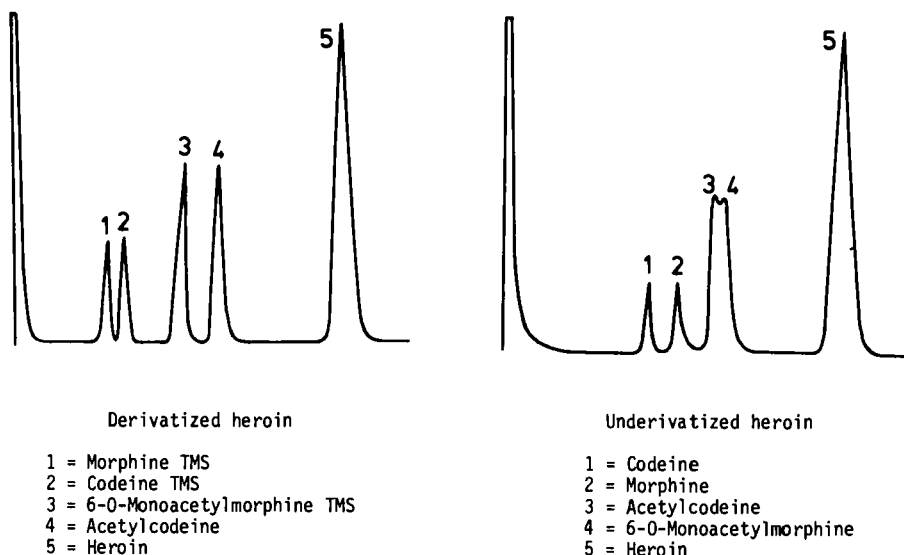


TABLE 14.14

RATIO OF BY-PRODUCTS IN HEROIN SAMPLES⁹²

Sample No.	Morphine	6-O-Monoacetyl- Morphine	Codeine	Acetyl- Codeine	Heroin hydro- chloride
1771	-	1.70	0.13	2.69	95.5
1772	-	1.81	0.09	2.85	95.2
1774	-	1.89	-	2.88	94.7
1775	0.03	1.90	0.06	2.97	95.0
1776	-	1.95	0.12	3.25	94.7
1777	-	1.80	-	3.01	95.2
1832	0.03	1.92	0.10	3.13	94.8
1833	0.03	2.40	0.30	3.35	93.2

Van der Slooten and Van der Helm⁹³ developed a similar method to be able to draw conclusions about the common origin or common trade route of different heroin samples by determining the ratio heroin:6-O-monoacetylmorphine:morphine. Other constituents of the samples such as amphetamines, quinine, methadone, cocaine and strychnine were determined in an analogous way. Because of the great variety of substances which may be present, three different packed columns (SE-30 3 %, QF-1 3 % and Apiezon L 10 % + KOH 2.5 %) were used for the identification.

In a study of procedures for the identification of heroin Clark⁹⁴ also used gas chromatography and separated heroin and 17 chemically closely related compounds on packed columns of OV-1 and OV-17 at 250°C. The retention times relative to heroin are given in Table 14.15.

TABLE 14.15

RETENTION TIMES OF HEROIN AND RELATED COMPOUNDS⁹⁴

Glass columns 6 ft by 4 mm I.D. at 250°C

Compound	3 % OV-1	3 % OV-17
Morphine	0.80	0.55
Dihydromorphinone	- *	0.70
Dihydromorphine	0.80	0.52
Codeine	0.61	0.49
Dihydrocodeine	- *	0.63
Ethylmorphine	0.65	0.47
Monoacetylmorphine	0.80	0.68
Monoacetyldihydromorphine	0.79	0.61
Acetylcodeine	0.77	0.64
Acetylmorphine	0.83	0.68
Propionylcodeine	0.94	0.81
Diacetylmorphine (heroin)	1.00 (3.95**)	1.00 (4.50**)
Pseudoheroin (acetyldihydromorphinoneenol acetate)	1.00	1.13
Propionylethylmorphine	0.99	0.85
Diacetyldihydromorphine	0.93	0.88
Monoacetylmonopropionyl dihydromorphine	1.12	1.09
Dipropionylmorphine	1.51	1.55
Dipropionyl dihydromorphine	1.34	1.32

*) Retention time could not be determined because of excessive peak symmetry, **) Retention time of diacetylmorphine (heroin) in minutes

In order to identify and determine 3-O-acetylmorphine in illicit heroin samples Moore and Klein⁹⁵ applied gas chromatography. 3-O-acetylmorphine results from an incomplete esterification of morphine with acetic anhydride and the amount may be of value for forensic purposes. Because of the very small amounts present in heroin, the compound was derivatized with heptafluorobutyric anhydride and gas chromatographed with a ⁶³Ni electron capture detector on a 3 % OV-17 on Gas Chrom Q packed column at 230°C using chlorpromazine as an internal standard. The heptafluorobutyric anhydride derivatives were extracted quantitatively from the reaction mixture with light petroleum and were stable for several hours in this solvent. However, it was recommended that upon formation, the analysis should be completed without delay. The analysis was carried out with 1-10 mg heroin samples and the amount of 3-O-acetylmorphine varied from 0.1 to 2 %, acetylcodeine from 3 to 15 %, morphine and codeine from 0.01 to 0.5 %.

In a review on the forensic identification of heroin Manura et al.⁹⁶ tested GLC and analyzed heroin and 56 morphine derivatives on four GC columns using 3 % OV-1, 3 % OV-17, 3 % OV-25 and 6 % Dexsil as stationary phases at column temperatures between 240°C and 280°C. The compounds analyzed and their retention times relative to heroin are given in Table 14.16.

With quantitative gas chromatography Han-Yong Lim and Sui-Tse Chow⁹⁷ analyzed more than a thousand illicit samples of heroin and found that all of them contained monoacetylmorphine, acetylcodeine and the diluent caffeine. The heroin content of most samples was within the range 30-50 % - in some cases even over 70 % - with corresponding low values for caffeine. The GLC was carried out on 1 m long packed columns with 3 % OV-17 on Gas Chrom Q at 235°C using codeine as internal standard - without derivatization. The standard deviation of samples in the range of 10.0 to 55.0 mg, containing about 36 % heroin, was for heroin 0.52, and the coefficient of variation 1.4 %.

A rapid and sensitive method to determine morphine, codeine and 6-O-monoacetylmorphine in

TABLE 14.16

RETENTION TIMES OF MORPHINE DERIVATIVES RELATED TO HEROIN ON FOUR GLC COLUMNS⁹⁶

- 1 = 3 % OV-1 on Chromosorb W, 80-100 mesh, glass column 1.2 m by 6.35 mm O.D. at 250°C
 2 = 3 % OV-17 on Chromosorb W, 80-100 mesh, glass column 1.2 m by 6.35 mm O.D. at 280°C
 3 = 3 % OV-25 on Gas Chrom Q, 80-100 mesh, stainless steel column 1.8 m by 3.18 mm O.D. at 240°C
 4 = 6 % Dexsil 400 on Gas Chrom Q, 80-100 mesh, stainless steel column 1.8 m by 3.18 mm O.D. at 240°C

Compound	Column			
	1	2	3	4
Morphine	0.56	0.60	-	-
Dihydromorphine	0.60	0.59	0.40	0.36
3-O-Acetylmorphine	0.73	0.74	0.67	0.80
3-O-Acetyldihydromorphine	0.71	0.69	0.63	0.82
6-O-Acetylmorphine	0.79	0.74	0.67	0.85
6-O-Acetyldihydromorphine	0.73	0.68	0.64	0.86
Heroin	1.00	1.00	1.00	1.00
Diacetyldihydromorphine	0.90	0.91	0.86	0.86
3-O-Propionylmorphine	0.88	0.86	0.84	1.05
3-O-Propionyldihydromorphine	0.94	0.83	0.79	0.96
6-O-Propionylmorphine	0.96	0.88	0.82	1.00
6-O-Propionyldihydromorphine	0.98	0.80	0.74	0.97
Dipropionylmorphine	1.63	1.48	1.54	1.66
Dipropionyldihydromorphine	1.48	1.35	1.26	1.40
3-O-Acetyl-6-O-propionylmorphine	1.31	1.23	1.23	1.29
3-O-Acetyl-6-O-propionyldihydromorphine	1.08	1.07	1.04	1.09
3-O-Propionyl-6-O-acetylmorphine	1.38	1.30	1.23	1.29
3-O-Propionyl-6-O-acetyldihydromorphine	1.13	1.09	1.05	1.12
3-O-Butionylmorphine	1.17	1.09	1.03	1.38
3-O-Butionyldihydromorphine	1.19	1.07	0.98	1.23
6-O-Butionylmorphine	1.17	1.09	1.02	1.27
6-O-Butionyldihydromorphine	1.14	0.95	0.88	1.10
Dibutionylmorphine	2.58	2.21	2.36	2.78
Dibutyonyldihydromorphine	2.25	1.85	2.28	2.64
3-O-Butionyl-6-O-acetylmorphine	1.75	1.56	1.59	1.70
3-O-Butionyl-6-O-acetyldihydromorphine	1.50	1.33	1.30	1.45
3-O-Acetyl-6-O-butyonylmorphine	1.58	1.48	1.51	1.66
3-O-Acetyl-6-O-butyonyldihydromorphine	1.33	1.31	1.24	1.36
3-O-Butionyl-6-O-propionylmorphine	2.17	1.89	1.88	2.12
3-O-Butionyl-6-O-propionyldihydromorphine	1.74	1.60	1.54	1.77
3-O-Propionyl-6-O-butyonylmorphine	2.13	1.85	1.86	2.12
3-O-Propionyl-6-O-butyonyldihydromorphine	1.70	1.58	1.51	1.71
Codeine	0.57	0.51	0.44	0.63
Dihydrocodeine	0.55	0.49	0.42	0.61
Acetylcodeine	0.77	0.69	0.63	0.67
Dihydroacetylcodeine	0.70	0.60	0.53	0.59
Propionylcodeine	1.02	0.83	0.77	0.85
Propionyldihydrocodeine	0.93	0.75	0.63	0.74
Butionylcodeine	1.30	1.04	0.96	1.10
Butionyldihydrocodeine	1.05	0.86	0.74	0.90
Ethylmorphine	0.64	0.55	0.46	0.69
Dihydroethylmorphine	0.61	0.54	0.43	0.65
Acetyl ethylmorphine	0.84	0.73	0.64	0.72
Acetyldihydroethylmorphine	0.75	0.65	0.54	0.62
Propionylethylmorphine	1.07	0.86	0.78	0.92
Propionyldihydroethylmorphine	0.95	0.75	0.64	0.76
Butionylethylmorphine	1.32	1.10	0.98	1.17
Butionyldihydroethylmorphine	1.14	0.89	0.75	0.94
Oxymorphone	0.86	-	-	-
Dihydromorphinone	1.11	-	-	-
Oxycodone	0.80	0.83	-	-
Dihydrocodeinone	0.66	0.65	0.61	0.69
Racemorphan	0.39	0.28	0.22	0.30

TABLE 14.16 (continued)

Compound	Column			
	1	2	3	4
Racemethorphan	0.30	0.21	0.14	0.19
Levallorphan	0.52	0.40	0.31	0.44
Apomorphine	0.55	-	-	-
Thebaine	0.77	0.82	0.84	0.73

illicit heroin was developed by Moore⁹⁸. After derivatization with heptafluorobutyric anhydride the fluorinated derivatives were extracted from an acetonitril-sodium bicarbonate solution into light petroleum in a one-step extraction procedure and gas chromatographed on a 3 % OV-17 column at 200-240°C. Morphine, codeine and 6-O-acetylmorphine were quantitated in heroin samples at levels as low as 0.001 %, 0.01 % and 0.001 % respectively. The gas chromatographic column was conditioned at 175°C for 1 h by injecting 5 x 5 µl of Silyl-8 (Pierce) and 5 x 5 µl of light petroleum containing 6-O-acetylmorphine (HFB) at a concentration of 5 mg/ml. The temperature was then increased to 285°C and maintained for 48 h. Minimum detectable quantity was ca. 20 pg for morphine, 80 pg for codeine and 100 pg for 6-O-acetylmorphine.

The content of morphine and 6-O-acetylmorphine in illicit heroin samples was determined by Machata and Vycudilik⁹⁹ by extractive propionylation in aqueous solution (disodium hydrogenphosphate buffer) with ethyl acetate as extractive solvent. Only the phenolic 3-OH-group of morphine reacts to 3-O-propionylmorphine; 6-O-propionylmorphine cannot be prepared by this method. On a 2.5 % OV-1 or SE-52 column these compounds were well separated, as shown in Table 14.17. The standard deviation and variation of the quantitative determinations using amitriptyline as an internal standard are given in Table 14.18.

TABLE 14.17

RETENTION INDEX (KOVATS) OF HEROIN AND RELATED COMPOUNDS IN ILICIT SAMPLES⁹⁹
On OV-1 and SE-52 at 250°C

	OV-1 column	SE-52 column
Caffeine	1818	1990
Amitriptyline (internal standard)	2240	2288
Codeine	2429	2501
Thebaine	2522	2629
6-Acetylcodeine	2535	2616
3-Propionylmorphine	2609	2701
Heroin	2638	2741
6-Acetyl-3-propionylmorphine	2722	2828
Papaverine	2808	2936

TABLE 14.18

STATISTICAL DATA FOR QUANTITATIVE DETERMINATIONS OF HEROIN AND RELATED COMPOUNDS⁹⁹

	% Standard deviation n = 10	Variation (95 %)
Codeine	6.9	15.6
6-Acetylcodeine	5.1	11.6
3-Propionylmorphine	6.0	13.6
Heroin	5.5	12.4
6-Acetyl-3-propionylmorphine	7.6	17.2

Van Vendeloo et al.¹⁰⁰ developed a gas chromatographic method for fingerprint analysis of illicit heroin samples, capable of detecting the main components: acetylcodeine, caffeine, codeine, heroin, 6-O-monoacetylmorphine, morphine and quinine in one run in 25 min. Heroin, morphine, codeine and caffeine could be quantified directly, 6-O-monoacetylmorphine and acetylcodeine were not fully separated. Quantitation of the latter two required acetylation of 6-O-monoacetylmorphine to heroin. A packed column of 1 % OV-1 on Chromosorb G HP was used and the analysis carried out at a column temperature of 275°C with amitriptyline as an internal standard, as⁹⁹.

Gough and Baker¹⁰¹ studied a number of conventional and modified stationary phases in order to find the best one for quantitative gas chromatography of heroin and structurally related compounds. Silanized OV-210 was found to be the most suitable for the separation of heroin, codeine, acetylcodeine, morphine and 6-O-monoacetylmorphine. It gave the best reproducibility of retention times and less losses of the compounds by adsorption. For quantitative analysis 2.8 m by 4 mm I.D. glass columns and Diatomite CLQ, 80-100 mesh, as solid support were used at a column temperature of 225°C. Despite the fact that some of the compounds, particularly morphine, suffered adsorption losses during gas chromatography, these losses were reproducible, and satisfactory quantitative data could be obtained, as shown in Table 14.19.

TABLE 14.19

PERCENTAGE COMPOSITION OF ILICIT HEROIN SAMPLES DETERMINED BY GLC¹⁰¹

Sample	Caffeine		Codeine		Morphine		Acetylcod.		Acetylmor.		Heroin		Amount inj.	
	Mean	True	Mean	True	Mean	True	Mean	True	Mean	True	Mean	True	Mean	True
1	3.7	4.5	6.9	6.9	-	-	-	-	11.0	10.2	78.4	78.4	2.68	2.65
2	4.0	4.5	13.8	13.7	-	-	-	-	17.1	17.3	65.1	64.5	2.68	2.69
3	18.7	19.5	8.1	7.5	-	-	-	-	16.8	16.6	56.4	56.4	2.48	2.47
4	19.0	20.9	17.5	16.0	-	-	-	-	16.5	17.7	47.0	45.4	2.31	2.29
5	27.9	28.0	27.9	27.4	-	-	-	-	-	-	44.2	44.6	1.17	1.22
6	14.8	15.8	47.5	48.4	-	-	-	-	37.7	35.8	-	-	3.10	3.03
7	15.4	15.6	48.7	47.6	-	-	35.8	36.8	-	-	-	-	3.07	3.08
8	15.3	15.7	48.2	48.2	-	-	19.0	18.5	17.5	17.5	-	-	3.16	3.05
9	19.0	19.1	58.9	58.4	-	-	22.0	22.5	-	-	-	-	2.63	2.51
10	4.5	4.7	4.2	4.9	9.3	9.7	14.7	14.6	9.9	9.7	57.4	56.4	2.64	2.57
11	4.7	4.8	9.4	9.9	13.9	14.7	7.3	7.5	16.4	15.1	48.3	48.0	2.56	2.52
12	18.0	18.6	4.5	4.8	9.6	9.7	14.8	14.5	15.4	14.7	37.7	37.7	2.60	2.58
13	17.5	18.1	9.0	9.4	9.3	9.4	22.0	21.2	14.8	14.3	27.4	27.6	2.67	2.65

Caffeine, Codeine, Morphine, Acetylcodeine, Acetylmorphine and Heroin in %, Amount injected in µg

The use of a stable-isotope labelled molecule is probably the closest approach to an ideal internal standard, because of the nearly identical physical and chemical properties between the molecules. Under most gas chromatographic systems, the compound and its deuterated analogue will co-chromatograph, with the distinction that the molecular ion in the fragmentation pattern will differ in mass by substituted deuterons. However, Jerpe et al.¹⁰² noticed a small difference in gas chromatographic retention times between the internal standard of deuterated heroin and heroin. The addition of three protons per acetyl group and increase in mass of 6 parts per 369 evidently changes the partition characteristics of deuter-

ated heroin enough to account for this slight separation (of four to six seconds on a 3 % OV-17 column, 4 ft long). The intensity of each specific ion fragment is recorded on a separate channel of a multiple recorder. Specially diluted solution concentrations are graphed to determine linearity of response within the magnitude of 1 to 100 ng of the sample injected on the column. Quantitation is achieved by comparing the relative peak areas.

Shui-Tse Chow¹⁰³ stated that the only physical method offering the necessary identification selectivity with quantitative capability for the gas chromatographic analysis of heroin in illicit samples is selected ion monitoring (SIM) mass spectrometry. Deuterated heroin and the ions m/e 369 and 327 for heroin and 375 and 331 of deuterated heroin were used as internal standards and the ions m/e 369 and 327 were quantified and calculated as heroin. The calibration curves for both m/e 375/369 and m/e 331/327 were linear within the concentrations studied. The gas chromatographic analysis was carried out on a 1.8 m by 6.35 mm O.D. glass column packed with 3 % OV-17 on Chromosorb W HP, 100-120 mesh, at a column temperature of 270°C. For GC-MS 1.8 m by 6.35 mm O.D. glass columns were used, packed with 3 % OV-1 on Chromosorb W HP, 100-120 mesh, and a column temperature of 250°C. The results obtained are summarized in Table 14.20.

TABLE 14.20

HEROIN PERCENTAGES DETERMINED BY GLC AND SIM¹⁰³

All results were the average of three injections

Sample	SIM		GLC	HPLC
	m/e 375/369	m/e 331/327		
1	39.4	40.1	39.4	38.8
2	39.2	40.5	38.9	39.3
3	39.7	41.1	40.3	39.5
4	39.8	40.8	40.0	38.7
5	40.3	41.3	39.7	38.6
Mean	39.6	40.7	39.6	38.9
Standard deviation	0.42	0.47	0.54	0.39
Coefficient of variation, %	1.06	1.17	1.36	1.01

In a study on heroin metabolism Elliot et al.¹⁰⁴ separated codeine, morphine, norcodeine, normorphine, 6-O-acetylmorphine, 3-O-acetylmorphine as trimethylsilyl derivatives and heroin on packed columns with SE-30 and QF-1, and assayed the amounts of morphine, 6-O-acetylmorphine and heroin in blood and urine samples, using methyl arachidate as an internal standard.

Whereas detection limits for heroin with conventional FID are reported as 0.05 mg/ml in blood from illicit preparations, a nitrogen specific detector can quantitate levels of 100 ng/ml - and detection limits can be as low as 20 ng/ml according to Smith and Cole¹⁰⁵. They determined heroin and its metabolite 6-O-acetylmorphine in blood after extraction and derivatization to its trifluoroacetate, to prevent adsorption during the gas chromatographic analysis when the free hydroxyl group was present. Ethylmorphine, derivatized in the same way was used as an internal standard.

To be able to determine heroin and its metabolite 6-O-acetylmorphine, morphine and normorphine in human urine, Yeh and McQuinn¹⁰⁶ made use of a fractionated extraction: Heroin was extracted with chloroform at pH 4.5, 6-O-acetylmorphine and morphine with ethylene dichloride containing 30 % isopropanol at pH 8.5 and normorphine at pH 10.4 with the same solvent. The extract was derivatized with trimethylsilylimidazole and chromatographed at 230°C

for the determination of 6-O-acetylmorphine and morphine, and at 220°C for normorphine and morphine. The overall recoveries for 6-O-acetylmorphine and morphine in concentrations of 2-15 µg/5ml of urine were 68-70 % and 60-62 % respectively. Heroin was determined by temperature programming from 200 to 250°C using cholestane as an internal standard. This substance was also used for the determination of morphine and normorphine, and for 6-O-acetylmorphine and heroin, whereas tetraphenylethylene was used as an internal standard for the determination of morphine and 6-O-acetylmorphine.

The metabolites of heroin in urine following intravenous administration of a single 10 mg dose were studied by Yeh et al.⁴² using TLC and column chromatography with the isolation of the various compounds and GLC or GC-MS of the silyl, trifluoroacetyl, acetyl or propionyl derivatives for the identification. Free morphine, 6-O-acetylmorphine, free normorphine, morphine 3-glucuronide, morphine 6-glucuronide, 6-O-acetylmorphine glucuronide and normorphine glucuronide were found as metabolites, morphine 3-glucuronide being the major metabolite (about 50 % of the administered dose of heroin). A 3 ft glass column with 3 % OV-17 on Gas Chrom Q and a 5 ft stainless steel column with 3 % SE-30 on Varaport were used by the gas chromatographic analysis. The retention times of some of the heroin metabolites are given in Table 14.21.

TABLE 14.21

RETENTION TIMES OF SOME HEROIN METABOLITES⁴²

	Trifluoroacetyl derivative 3 % OV-17 210°C	Trimethylsilyl derivative 3 % OV-17	
		220°C	230°C
6-O-Acetylmorphine	6.9 min	-	7.1 min
Codeine	5.8 -	-	-
Morphine	3.8 -	7.4 min	4.3 -
Norcodeine	9.1 -	-	-
Normorphine	6.2 -	8.8 -	-

14.1.2. Capillary columns

14.1.2.1. Morphine

Capillary gas chromatography of morphine and codeine was described by Christophersen and Rasmussen¹⁰⁷ in connection with their studies on flash heater derivatization of drugs for gas chromatographic analysis. Derivatization was found necessary for relative polar compounds such as morphine and codeine because of the often undesirable adsorption observed with glass capillary columns. Trimethylsilylation was performed with N,O-bis-(trimethylsilyl)acetamide and ethylmorphine was used as an internal standard for the quantitative determinations on a 20 m by 0.35 mm I.D. glass capillary, wall coated with SE-30. Calibration graphs for concentrations of 1-10 µg/ml in ethylacetate were constructed. The data obtained from the reproducibility test showed that at 5.0 µg/ml the relative standard deviation was 1.5 % for codeine and 4.3 % for morphine.

Huhtikangas et al.¹⁰⁸ used also glass capillary columns for quantitative determination of morphine in plasma levels. A relative simple method was developed based on extractive alkylation of morphine with pentafluorobenzyl bromide. A glass capillary, 18 m by 0.35 mm I.D. coated with OV-225, and nalorphine as internal standard were used. The detection limit with FID

was 2-5 ng, with ECD about 5 pg.

Edlund¹⁰⁹ described a method for simultaneous determination of morphine, 6-O-acetylmorphine and codeine in human plasma or blood. The samples were buffered to pH 9 and extracted on silica columns, cleaned by extraction and finally acylated with pentafluoropropionic anhydride. The derivatives formed were separated on a glass capillary column (25 m by 0.36 mm I.D., coated with OV-1) at 220°C. The falling needle injection and electron capture detection were used. Although degradation of the solutes was observed, the degradation observed was very reproducible, so that quantitative analyses could be carried out in spite of the degradation. The author recommended regular control and calibration in order to obtain reliable results.

Because of adsorption and degradation problems when using glass capillary columns for the analysis of morphine and codeine, Plotczyk¹¹⁰ preferred fused silica columns to the analysis of underivatized drugs, *i.a.* codeine. He used cold on-column injection and cross-linked polysiloxane deactivated columns and obtained linear quantitation from 1 to 100 ng with precisions of 0.1-2 % for some of the drugs analyzed.

14.1.2.2. Heroin

A number of heroin seizures in Münsterland (G.F.R.) were analyzed by Bohn et al.¹¹¹ with glass capillary gas chromatography. With a 12 m long column by 0.3 mm I.D. coated with Triton X 350 and temperature programming (200-250°C) a good separation was obtained for heroin, 6-O-acetylmorphine, morphine, acetylcodeine, caffeine and the internal standard, dotriacontan. The heroin samples contained heroin (19.1-58.2 %), 6-O-acetylmorphine (1.0-7.3 %), acetylcodeine (2.4-9.8 %), caffeine (26.5-64.3 %) and strychnine (0-1.26 %).

Glass capillary gas chromatography was used by Christophersen and Rasmussen¹⁰⁷ also for heroin. Whereas morphine, codeine, mono-acetylmorphine and ethylmorphine were derivatized with N,O-bis(trimethylsilyl)acetamide with flash heater derivatization, heroin was not derivatized, but it was well separated from the other compounds mentioned, as well as caffeine and strychnine, which were found in the heroin samples analyzed.

In a paper on profiling heroin samples by capillary chromatography for forensic application Neumann and Gloger¹¹² used glass capillary columns (25 m by 0.2 mm I.D.) coated with SE-54, and FID or NFID. The trace impurities of the heroin samples were extracted with toluene and derivatized with N-Methyl-N-trimethylsilyltrifluoroacetamide or N,O-bis(trimethylsilyl)acetamide. Remarkable differences were observed for samples from various origin, *i.a.* Turkey, Malaysia and Lebanon. Compounds such as acetylthebaol, diacetylnorcodeine, 4-O-acetyl-3,6-dimethoxy-5-(2-N-methylacetamido)-ethylphenanthrene and meconin were detected in varying amounts in the different heroin samples. The "fingerprinting" capillary gas chromatography of illicit heroin samples seems to have distinct advantage over most of the methods used so far for the forensic comparison of such samples.

The introduction of fused silica capillary columns opened new ways in the forensic analysis of heroin samples. Demedts et al.¹¹³ made use of a CP-Sil 5 fused silica column permanently deactivated with a polysiloxane at high temperature and a NP-detector and carried out determinations of heroin in the nanogram range without derivatization. Down to 250 pg heroin was detectable. Standard deviation and coefficients of variation were comparable to previously used packed columns. Acetylcodeine and 6-monoacetylmorphine, which could not be separated on the CP-Sil 5 column, were separated on a CP-Sil 8 column. The columns were 25 m by

0.32 mm or 0.22 mm I.D. Temperature programming was used: 200°C for 2 min, then 5°C/min to 260°C (held for 1 min), then 10°C/min (held for 1 min). Helium was used as carrier gas and diacetylnalorphine as internal standard. The reproducibility data of the method are shown in Table 14.22.

TABLE 14.22

REPRODUCIBILITY DATA FOR DETERMINATION OF HEROIN¹¹³

Within-run data and day-to-day precision and % heroin found

	Sample Nr.	Simulated heroin samples		Illicit heroin sample
		+ 40 %	+ 70 %	
Within-run	1	34.31	65.45	47.04
	2	35.10	63.71	46.17
	3	34.17	67.27	47.70
	4	34.30	65.00	46.42
	5	33.82	63.71	46.20
Day-to-day	6	32.64	64.81	45.92
	7	32.94	63.93	46.66
	8	34.31	64.02	47.85
	9	35.46	63.17	47.06
	10	34.45	64.12	47.12
Average		34.15	64.52	46.81
Standard deviation		0.86	1.18	0.65
Variation %		2.52 %	1.84 %	1.39 %

TABLE 14.23

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF OPIUM ALKALOIDS

Column	Solid support mesh	Stat.phase	%	Temperature	Comp.Preparation	Ref.
glass, 6 ft x 3 mm I.D.	GA ABS + PEG + NPE	SE-30	2	207°C	alk. s.	1
s.s., 6 ft x 2 mm I.D.	CW 100-120	SE-30		235-270°C	alk. s. tox.	2
glass, 6 ft x 3 mm	CG 100-120 acyl.	SE-52		250°C	mo.qnt.ng.fb.	3
glass, 6 ft x 4 mm I.D.	CW 80-100	SE-30	2-3	204°C	alk.s.	4
glass, 6 ft x 4 mm I.D.	GP 100-140	SE-30	1	150-250°C pr	alk.qnt.0	5
6 ft x 8 mm	GP 100-140	SE-30	1	185°C	alk.s.	6
s.s., 5 ft x 0.093 in	CW AH 60-80	SE-30	5	210°C 270°C	alk.s.tox.	7
glass, 6 ft x 4 mm I.D.	Ana ABS 100-120	SE-30	1	200°C 250°C	alk.s.tox.	8
glass, 3 ft x 3 mm O.D.	GP S 100-120	HI-EFF 8B	1	220°C 250°C	alk.s.tox.	9
s.s., 1.8 m x 3 mm	CG AHS 80-100	SE-30	3	210-280°C pr		
s.s., 1.5 m x 3 mm	Aer 30 80-100	OV-1	3	200-260°C pr	alk.O.s.her.	10
s.s., 1.0 m x 3 mm	Aer 30 80-100	OV-1	2	205°C	mam	
2 m	CG AW 80-100	SE-30	0.2			
1 m	CG AW 80-100	OV-17			alk.s. RI	11
glass, 1.5-2 m x 2-4 mm I.D.	CW, CG, Cel. Diat.	SE-30	2-3		alk.s. RI	12
glass, 3 ft x 6 mm	Sup. 01-1953	OV-17	3	210°C	mo.co.der.s.	13
glass, 6 ft x 7 mm I.D.	GS S 80-100	SE-30		215°C	alk.ocd.	14
glass, 6 ft x 3 mm I.D.	GS ABS 80-100	SE-30		215°C	alk.ocd.	15
glass, 6 ft x 3 mm I.D.	GP ABS PEG 100-140	SE-30	1.15	175°C 225°C	alk.s.	16

TABLE 14.23 (continued)

Column	Solid support	Stat.phase	%	Temperature	Comp.Preparation	Ref	
glass, 1 m x 3 mm I.D.	CW AWS 100-120	OV-17	2.9	205°C	mo.TMS qnt.ur.	17	
glass, 6 ft x 2 mm I.D.	Sup. 80-100	OV-22	3	215°C	mo.co.nlp.PFP ecd.	18	
glass S, 5 ft x 2 mm I.D.	GQ 100-120	OV-17	3	215°C	mo.PFP qnt.bi. ecd.	19	
glass, 3 ft	Var. 100-120	SE-30	3	205°C	mo.co.met.ox. ur.	21	
glass, 3 ft	GQ 60-80	OV-17	3	205°C			
glass, 6 ft	GQ 60-80	OV-17	3	200°C			
				250°C			
glass 2 m x 2 mm I.D.	Sup. 80-100	SE-30	3	210°C	220°C	mo.co.TFA,HFA ocd. qnt.	22
glass, 1.3 m x 3 mm I.D.	Sup. 80-100	SE-30	3	200°C	mo.co.etmo.HFA qnt. ocd.		23
glass, 1.8 m x 2 mm I.D.	GQ 100-120	Poly-A 103	3	230°C	mo.co.qnt.ur.	24	
glass, 1.8 m x 2 mm I.D.	CW HP AWS 80-100	OV-25	3	240°C			
glass, 6 ft x 6 mm O.D.	GQ 100-120	SE-30	3	205°C	alk.ab.ur.	26	
glass, 6 ft x 2 mm I.D.	GW 80-100	OV-1	3	240°C	mo.co.id.O.HS	28	
glass, 6 ft x 2 mm I.D.	GW HP 80-100	OV-101	3	250°C	id.O.GC-HS	29	
glass S, 6 ft x 4 mm I.D.	GP AW 80-100	SE-30	5	240°C	co.met.qnt.ser. ur.	30	
glass S, 6 ft x 6 mm	Dia.S 60-80	SE-30	3.8	225°C	mo.co.TMS qnt.	31	
glass, 4 ft x 4 mm I.D.	GP ABS PEG. 60-80	SE-30	4	183°C	mo.TMS qnt.O.	32	
0.65 m x 3 mm I.D.	Aer. 100-120	SE-30	5	200°C	mo.co.TMS qnt. pop.	33	
glass, 4 ft x 4 mm I.D.	Dia.S 80-100	SE-30	3.5	200°C	240°C	alk.fb.qnt.O.	34
glass, 1.5 m x 2.3 mm I.D.	CG HP AWS 80-100	HI-EFF 88	0.75	150-235°C	pr	alk.fb.qnt.O.	35
glass, 6 ft x 2 mm I.D.	CW AWS 80-100	SE-30	5	>1+1 250-280°C	pr	alk.fb.qnt.O.	36
	Var.30 80-100	OV-17	3				
glass, 6 ft x 2 mm	GQ 80-100	OV-17	3	240°C	mo.co.TMS qnt. O.	37	
glass, 6 ft x 2 mm	GQ 100-120	UCW-98	3.8	230°C			
glass, 1.5 m x 4 mm I.D.	GQ	OV-225 + OV-25	1	210°C	mo.TMS qnt.O.	38	
		Dex.300	3	250°C	mo.TMS qnt. ocd.	39	
glass, 1.8 m x 4 mm I.D.	Shimalite AWS	OV-1	1.5	210°C	mo.TMS qnt.ur.	40	
-	80-100	OV-17	1.5	220°C			
-	-	SE-30	1.5	200°C			
-	-	QF-1	1.5	170°C			
-	-	XE-60	1.5	195°C			
glass, 3 ft x 2 mm	GQ 60-80	OV-17	3	210-220°C	her.met.TFA, TMS qnt.	42	
s.s., 5 ft x 2 mm	Var. 100-120	SE-30	3	230°C	mo.TMS qnt.ur.	43	
s.s., 1.5 m x 2 mm I.D.	CW 80-100	Dex.	3	250°C			
glass, 0.9 m x 4 mm I.D.	CG AWS 100-120	JXR + CDMS	0.35 0.35	210°C	mo.TMS qnt.ur.	44	
glass, 6 ft x 2 mm I.D.	GQ 100-120	OV-17	1	228°C	deu.mo.HFB, co. TFA. qnt.MF	45	
glass, 2 m x 2 mm I.D.	GQ 100-120	OV-17	3	230°C	deu.mo. qnt. ur. MF	46	
s.s., 5 ft x 3 mm O.D.	GQ 100-120	OV-1	3	215°C	mo. TMS qnt.bi.	47	
6 ft	CW HP 80-100	OV-17	3	235°C	mo.qnt. der.bi.	48	
glass S, 3 m				215°C	mo. PFA. pl.	49	
glass, 0.5 m x 6 mm O.D.	GQ	SE-30		210°C	deu.mo.TFA qnt. br.	50	
glass, 3 ft x 4 mm I.D.	Sul. AWS 80-100	OV-17	3	220°C	mo.TFA qnt.ur.	51	
glass	GQ 100-120	OV-17	3	240°C	mo.co. TMS qnt.	52	
-	CW HP 80-100	UCW-98	3.8	240°C	pmt.		
glass S, 1.8 m x 2 mm I.D.	GQ 100-120	OV-17	3	215°C	mo.co.PFP qnt. pl.bi.	53	
glass S, 213 cm x 4 mm I.D.	Diat.C S	OV-17	2	245°C	265°C	mo.PFB,TFA qnt.	54
glass, 4 ft x 2 mm I.D.	CW HP 100-120	OV-1	3	240°C	pl. MF	mo.der.qnt.bi.	55

TABLE 14.23 (continued)

Column	Solid support	Stat.phase	%	Temperature	Comp.Preparation	Ref
glass, 1.2 m x 3 mm I.D.	CW HP 100-120	OV-17	3	240°C	moder.qnt.bl	56
90 cm x 2 mm I.D.	Sup. 100-120	OV-101	3	205-240°C	moder.qnt.bl	57
glass, 4 ft x 4 mm I.D.	GP ABS 60-80	SE-30	4	180°C	npa. qnt.0.	58
	PEG					
glass, 1.8 m x 2 mm I.D.	GQ S 80-100	OV-17	1	275°C	pa.qnt.pl.	59
glass, 0.91 m x 6.4 mm I.D.	Dia.S 80-100	Li.W.-98	3.8	140-275°C	co.qnt.prep.	60
0.0.D.						
glass, 1.0 m x 6.4 mm I.D.	CW AWS	OV-17	3	240°C	co.qnt.prep.	61
0.0.D.						
s.s., 6 ft x 3.1 mm I.D.	CW AW	SE-30	10	195-260°C	co.qnt.prep.	62
s.s., 6 ft x 3 mm I.D.	CW AWS 80-100	UCC-W 982	10	240°C	co.qnt.prep.	63
glass, 0.9 m x 6.4 mm I.D.	Dia. 80-100	Li.W.-98	3.8	145-255°C	co.qnt.prep.	64
glass, 1.0 m x 3 mm I.D.	Sup. 100-120	OV-17	3	250°C	co.norco.qnt.	65
0.0.D.					bi.	
glass S, 1.8 m x 2.5 mm I.D.	Ana.A 90-100	XE-60	2	230°C	co.qnt.pl.	67
0.0.D.						
glass, 3 ft x 2 mm I.D.	GQ 100-120	OV-17	3	100-240°C	mo.co.qnt.bi.	68
s.s., 7 ft x 3 mm I.D.	GQ 100-120	OV-17	3	260°C	th.qnt.P.b.	69
glass, 1.5 m x 4 mm I.D.	CP 100-120	SE-30	10	290°C	th.qnt.P.b.	70
0.0.D.						
glass, 6 ft x 3 mm I.D.	CG AWS 100-120	OV-17	2	270-290°C	alk.qnt.P.b.	71
glass, 4 ft x 3.5 mm I.D.	GQ 100-120	OV-17	3.8	260°C	th.qnt.P.b.	72
0.0.D.						
glass, 5 ft x 4 mm I.D.	CW AWS 80-100	OV-17	2	270°C	th.qnt.pm.	73
glass	GQ 100-120	OV-17	3	270°C	th.qnt.P.b.	74
glass, 6 ft x 3 mm I.D.	GQ 100-120	OV-17	2.5	270°C	th.qnt.P.b.	75
glass S, 1.5 m x 4 mm I.D.	GQ 100-120	OV-17	2	270°C	th.qnt.P.b.	76
0.0.D.						
glass, 1.5 m x 4 mm I.D.	GQ 100-120	OV-17	3	260°C	th.qnt.P.b.	77
glass,	GQ 100-120	OV-17	2		th.qnt.P.b.	78
glass, 1 m x 3 mm I.D.	GQ 100-120	OV-17	3	290°C	pa.qnt.bl.	79
glass, 2 m x 3 mm I.D.	GQ AWS 100-120	OV-17	3	270°C		
glass cap. 30 m x 0.25 mm I.D.		SE-30		225°C	pa.qnt.bl.	80
mm I.D.					MF	
glass, 2 m x 3 mm I.D.	GP 100-120	OV-1	1	225°C		
glass, 1.2 m x 3 mm I.D.	GQ 80-100	OV-17	3	265°C		
0.0.D.					pa.qnt.bl.	81
glass, 1.2 m x 2 mm I.D.	CW HP 100-120	OV-101	2	275°C		
0.0.D.						
glass, 2 m x 32. mm I.D.	CW 60-80	SE-30	2	230°C	et.mo.qnt.	82
0.0.D.					prep.	
glass, 6 ft x 3 mm I.D.	CW HP 80-100	OV-17	3	270°C	ant.ur.	83
0.0.D.	GQ 100-120	OV-17	3	260°C	apomo.met.qnt.	84
0.0.D.					ser.	
glass, 6 ft x 3 mm I.D.	GQ 100-120	OV-17	3	220°C 260°C	apomo.TMS qnt.	85
0.0.D.					ur.	
glass S, 76 cm x 4 mm I.D.	CW HP 100-120	OV-17	3	190°C	apomo.qnt.pl.	86
0.0.D.						
glass, 5 ft x 4 mm I.D.	CW AW 80-100	CHDS	3	250°C	her.cg.qnt.	87
s.s., 6 ft x 3 mm I.D.	CW AWS 60-80	SE-30	5	200°C	her.qnt.	88
6 ft x 2 mm I.D.	CW HP 80-100	OV-1	3	235°C	her.qnt.	89
glass, 6 ft x 4 mm I.D.	CW HP 80-100	OV-1	2	275°C	her.qnt.	90
s.s., 6 ft x 3 mm I.D.	WHP 100-120	OV-17	3	260°C	her.qnt.id.IR	91
glass, 6 ft x 3 mm I.D.	GQ 100-120	OV-25	3	240°C	mo.co.acco.	92
0.0.D.					mam.TMS qnt.	
glass, 1.2 m x 3 mm I.D.	CW HP 80-100	SE-30	3	235°C		
glass, 2.1 m x 3 mm I.D.	GQ	QF-1	3	235°C	her.qnt.	93
glass, 0.9 m x 3 mm I.D.	CW AWS 80-100	Apiezon	10	150°C		
	+ KOH		2.5			

TABLE 14.23 (continued)

Column	Solid support	Stat.phase	%	Temperature	Comp.Preparation	Ref
glass, 6 ft x 4 mm I.D.		OV-1	3	250°C	her.cg. mam.	94
-		OV-17	3	250°C	HFB qnt.	
glass, 6 ft x 4 mm I.D.	GQ 100-120	OV-17	3	230°C	mam.HFB.qnt.	95
glass, 3 ft x 2 mm I.D.	GQ 80-100	OV-17	3	180°C pr	her. ECD, MS	
glass, 1.2 m x 6.35 mm	CW 80-100	OV-1	3	250°C		96
- O.D.	-	OV-17	3	280°C		
s.s., 1.8 m x 3.18 mm	GQ 80-100	OV-25	3	240°C	56 mo.der. s.	
- O.D.	-	Dex.400	6	240°C		
glass, 1 m x 6.35 mm	GQ 80-100	OV-17	3	235°C	her.cg.qnt.	97
- O.D.						
glass, 1.8 m x 4 mm	GQ 100-120	OV-17	3	200-220°C	co.	98
I.D.				220-240°C	mo.mam HFB qnt.	
glass, 2 m x 2 mm I.D.	CG AWS 80-100	SE-52	2.5	240-280°C pr	mo.mam.prop.	99
-	-	OV-1	2.5		der. qnt.her.	
glass, 1.8 m x 2 mm I.D.	CG HP 80-100	OV-1	1	235°C	fprint. her.	100
glass, 2.8 m x 4 mm I.D.	Diat.CLQ 80-100	OV-210 S	3	225°C	co.mo.acco.	101
					mam.her.qnt.	102
glass, 4 ft x 6 mm	CW HP 80-100	OV-1	1	230°C	her.qnt.MF	102
glass, 1.8 m x 6.35 mm	CW HP 100-120	OV-1	3	250°C	her.qnt.	103
- O.D.	-	OV-17	3	270°C	SIM	
s.s., 5.3 ft x 2 mm I.D.	CW HP 100-120	SE-30	3	218°C	her.met TMS	104
s.s., 9 ft x 2 mm I.D.	CW HP 100-120	QF-1	3	218°C	qnt.ur.	
glass, 213 cm x 4 mm	Diat.C AW 100-120	OV-17	2	250°C	her.mam.TFA.	105
I.D.					qnt.bl. ND	
glass, 3 ft x 2 mm	GQ 60-80	OV-17	3	200-250°C pr	her.	106
				230°C	mo.mam.TMS	
				220°C	mo.normo.TMS	107
glass cap. 20 m x 0.35 mm I.D.		SE-30		50-250°C pr	qnt.ur.	
					mo.co.TMS qnt.	107
glass cap. 18 m x 0.35 mm I.D.		OV-225		50-230°C	ocd.	108
glass cap. 25 m x 0.36 mm I.D.		OV-1		220°C	mo.PFB qnt.pl.	
					mo.co.mam.	109
f.sil cap. 25 m x 0.32 mm I.D.		SE-54		80-280°C pr	PFP. qnt.bi.	
glass cap. 12 m x 0.3 mm I.D.		Tr.X305		200-250°C pr	co.	110
					mam.acco.ca.	111
glass cap. 25 m x 0.2 mm I.D.		SE-54		150-300°C pr	str. her.qnt.	
f.sil cap. 25 m x 0.32 mm I.D.		CP-Sil 5		200-300°C pr	prof.her.	112
					her.tox.qnt.	113
					ND	

TABLE 14.24

OPIUM ALKALOIDS - LIST OF ABBREVIATIONS

ABW = acid, base washed

ab = abuse

acco = acetylcodeine

alk = alkaloid

Ana = Anakrom

angt = narcotic antagonist

apomo = apomorphine

AW = acid washed

bi = biological material

bl = blood

br = brain

CA = Chromosorb A

ca = caffeine

cap = capillary

Cel = Celite

CG = Chromosorb G

cg = congener

co = codeine

CHDS = cyclohexane dimethanol succinate

CG = Chromosorb G

CW = Chromosorb W

der = derivative

deu = deuterium

Dex = Dexsil

Dia S = Diatoport S

Diat = Diatomite

ECD = electron capture detector

etmo = ethylmorphine

fb = free base

fprint = finger print

f.sil = fused silica

ft = feet

GA = Gas Chrom A

GP = Gas Chrom P

GQ = Gas Chrom Q

GS = Gas Chrom S

her = heroin

HFA = heptafluoroacetyl

```
in = inch
IR = infra red
Li = Linde
mam = monoacetylmorphine
met = metabolite
MF = mass fragmentography
MS = mass spectroscopy
ND = nitrogen detector
NGS = neopentyl glycol succinate
nlp = nalorphine
norco = norcodeine
normo = normorphine
nos = noscapine
npa = non phenolic alkaloids
NPE = nonyl phenoxyethylene oxyethanol
O = opium
ocd = on-column derivatization
OD = outside diameter
ox = oxydation product
```

pa = papaverine
P.b. = *Papaver bracteatum*
 PEG = polyethylene glycol
 PFB = pentafluorobenzyl
 PFP = pentafluoropropionyl
 pl = plasma
 pm = plant material
 pmt = post mortem tissue
 pop = poppy
 pr = (temperature) programming
 prof = profiling
 prop = propionyl
 qnt = quantitative
 RI = retention index
 S = silanized
 s = separation
 ser = serum
 SIM = selective ion monitoring
 s.s = stainless steel
 str = strychnine
 Sup = Supelcoport
 TFA = trifluoroacetyl
 th = thebaine
 TMS = trimethylsilyl
 tox = toxicology
 TrX305 = Triton X 305
 ur = urine
 Var = Varaport

TABLE 14.25

DERIVATIZATION OF OPIUM ALKALOIDS

SILYLATION

1. With hexamethyldisilazane

Morphine (10-25 mg) is dissolved in 2 ml of dry pyridine followed by 1 ml of hexamethyldisilazane. The vial is stopped with a polyethylene stopper and set aside at room temperature for 16-18 hours. The solution is gas chromatographed directly.³²

2. With hexamethyldisilazane + trimethylchlorosilane

Morphine (1 mg) is dissolved in a solution of 0.75 ml of pyridine, 0.25 ml of trimethylchlorosilane and 0.25 ml of hexamethyldisilazane. A 0.25 μ l sample is injected for gas chromatography³¹.

3. With N,O-bis(trimethylsilyl)acetamide

50 μ l of a stock solution of morphine, codeine and/or nalorphine (0.1 mg/ml) is evaporated in a vial, the residue taken up in 100 μ l of a 40 % solution of N,O-bis(trimethylsilyl)acetamide in pyridine and 1 μ l is injected into the gas chromatograph⁵².

Morphine and/or monoacetylmorphine is solved in 1.0 ml of pyridine and 0.5 ml of N,O-bis-(trimethylsilyl)acetamide in a vial. The vial is capped and the sample and reagents are mixed by tapping about 15 second. The sample is allowed to stand 5 minutes before injection of 2 μ l⁸⁸.

TABLE 14.25 (continued)

One ml of N,O-bis(trimethylsilyl)acetamide is added to 0.07-3.4 mg of apomorphine in a Microflex tube fitted with Teflon-lined cap. After shaking gently for 20 minutes 1 μ l is used for gas chromatography⁸⁵.

4. With N,O-bis(trimethylsilyl)trifluoroacetamide + 10 % trimethylchlorosilane

Add to morphine, codeine, normorphine, norcodeine, morphine-N-oxyde and/or morphine-N-methyl iodide, placed in an acylation tube 0.05 ml acetonitrile and 0.05 ml of N,O-bis(trimethylsilyl)trifluoroacetamide containing 10 % trimethylchlorosilane. Cap the tube and shake in a Vortex mixer for about 10 seconds. Heat at 60-70°C for 30 minutes and inject 1 μ l into the gas chromatograph²¹.

5. With trimethylsilylimidazole

Morphine and/or normorphine (0.5-1 mg) is mixed in a sealed acylation tube with 50 μ l of 25 % trimethylsilylimidazole in pyridine and heated in an oil bath at 90-95°C for 1 hour. One μ l is injected for gas chromatographic analysis¹⁰⁶.

ON COLUMN SILYLATION

1. With trimethylsilylimidazole

One μ l of a morphine solution in ethylacetate is drawn into a Hamilton syringe 750 N followed by 2 μ l of trimethylsilylimidazole - and injected into the gas chromatograph³⁹.

ACYLATION

1. With acetic anhydride

Morphine (normorphine) (0.5-1 mg) is dissolved in 0.2 ml of acetic anhydride and 0.1 ml of pyridine in a sealed acylation tube and heated at 60-70°C for $\frac{1}{2}$ hour. The excess of acetic anhydride is removed by evaporation and the residue dissolved in 50 μ l of ethyl acetate. One μ l of the solution is injected into the gas chromatograph¹⁰⁶.

2. With trifluoroacetic anhydride

Morphine (25 ng-5 mg) is solved in 0.1 ml ethyl acetate and 0.1 ml trifluoroacetic anhydride in a tube. The tube is tightly capped and shaken in a Vortex mixer. Then it is placed in a water bath of 50°C for 20 minutes, after which the content is evaporated to dryness under a gentle stream of filtered air at 50°C. The residue is solved in 0.1 ml ethyl acetate and 1-5 μ l used for gas chromatographic analysis⁵¹.

3. With pentafluoropropionic anhydride

Morphine (1 ng-1 μ g) is solved in 50 μ l dry ethyl acetate and 100 μ l pentafluoropropionic anhydride is added. The tightly stoppered tube is placed in an oven at 60°C for 30 minutes. Excess of reagent is removed by a gentle stream of dry nitrogen and the residue dissolved in 50 μ l dry ethyl acetate. Of this solution 1-2 μ l is injected for gas chromatographic analysis¹⁹.

A solution containing 1.0 ng to 1.0 μ g free base of either morphine or codeine is evaporated to dryness in a 15 ml conical test tube which has previously been treated with a 5 % (v/v) solution of Dri-Film SC-87 in toluene (Pierce Chemical Co.). To the tube 100 μ l of

TABLE 14.25 (continued)

glass distilled benzene and 100 μ l of pentafluoropropionic anhydride is added. The tube is capped and then allowed to react for 25 minutes at 70°C. After reaction the solvent with the reagent is evaporated to dryness at room temperature under a stream of dry nitrogen. The sample is taken up in ethyl acetate and 1 μ l, containing 5-1000 pg per μ l is injected into the gas chromatograph¹⁸.

ON COLUMN ACYLATION

1. With acetic anhydride

The injection of the sample of morphine (0.5 % solution in methanol, ethyl acetate or tetrahydrofuran) (6 μ l) is followed within 5 second of that of 5 μ l of acetic anhydride¹⁴.

2. With propionic anhydride

The same technique as described above but using 5 μ l of propionic anhydride^{15,55}.

3. With trifluoroacetylimidazole or heptafluorobutyrylimidazole

One μ l of a solution of morphine or codeine (0.1-2.5 mg/ml) in ethyl acetate is drawn into a Hamilton 701-N syringe followed by 2 μ l of the acylating reagent. The mixture is injected into the gas chromatograph²².

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Chapter 15

APORPHINE ALKALOIDS

15.1 Aporphine alkaloids	147
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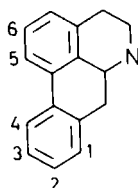
15.1 APORPHINE ALKALOIDS

Arndt et al.¹ gas chromatographed 27 aporphine derivatives, naturally occurring alkaloids and synthetic derivatives, in order to study the correlation between molecular weight and/or other structural features and retention times. A packed 0.3 % SE-30 on glass micro beads column and two column temperatures, 235°C and 250°C, were used for the investigations. A number of the phenolic alkaloids could not be eluted from the column and they were converted to their ethyl esters. Even after esterification some of them, boldine, corytuberine and hermandine failed to give an observable response. Other aporphine derivatives behaved in a similar way. The results in Table 15.1 show that there is no linear relationship between the relative retention times and molecular weight of the compound investigated.

TABLE 15.1

RELATIVE RETENTION TIMES OF APORPHINE DERIVATIVES VS. 2-CHLOROAPORPHINE¹

2-Chloroaporphine has a retention time of 3.7 and 1.9 min at 235°C and 250°C, respectively; glass column 2 m by 5 mm I.D., 0.3 % SE-30 on micro glass beads.



	Substituents		RRT		Molecular weight
	Ring	Nitrogen	235°C	250°C	
1.	3-CF ₃	Me	0.49		303
2.	3-F	Me	0.52		253
3.	3-CH ₃	Me	0.73		249
4.	1-CH ₃	Me	0.78		249
5.	1-6 H	Me	0.80		235
6.	1-Cl	Me	0.89		269.5
7.	3-Cl	Me	0.99		269.5
8.	2-Cl	Me	1.00		269.5
9.	3-CF ₃ -5,6-di-OCH ₃	Me	1.02		363
10.	3-OCH ₃	Me	1.20		265
11.	2-OCH ₃	Me	1.21		265
12.	5,6-di-OCH ₃	Me	1.26		295
13.	3,4-di-OCH ₃	Me	1.29		295
14.	3-OCH ₃ -4-OCH ₃	Me	1.30		309
15.	5,6-di-OCH ₃	H	1.35		281
16.	2-OC ₂ H ₅	Me	1.38		279

TABLE 15.1 (continued)

Ring	Substituents	Nitrogen	RRT		Molecular weight
			235°C	250°C	
17.	5,6-di-OCH ₃	Ct	1.44		309
18.	3,4-di-OC ₂ H ₅	Me	1.52		323
19.	3,4,6-tri-OCH ₃ -5-OC ₂ H ₅	Me		2.04	369
20.	3,4,6-tri-OCH ₃	Me	2.36		325
21.	3-OCH ₃ -4-OH	Me	2.44		281
22.	3,4-benzo	Me	2.50		285
23.	3-OCH ₃ -4-OC ₂ H ₅ -5,6-CH ₂ O ₂	Me		2.54	353
24.	3,4-di-OCH ₃ -5,6-CH ₂ O ₂	Me		2.78	339
25.	2,3,5,6-tetra-OCH ₃	Me		4.37	355
26.	5,6-di-OCH ₃	Me	4.52		323
27.	2-OC ₂ H ₅ -3,5,6-tri-OCH ₃	Me		30	397

Cashaw et al.² carried out a comparative study on the gas chromatographic properties of the TMS derivatives of some naturally occurring aporphines and tetrahydroprotoberberines (THPB) and the TMS derivatives of their demethylated products on OV-1 and OV-17. A better separation of the silyl derivatives of the aporphine and the THPB alkaloids was achieved on the OV-1 column. A direct correlation of the number of TMS groups or molecular weight with the elution pattern of the aporphines was not apparent. Boldine, with two TMS groups, had a greater retention time than silylated 1,2,9,10- and 1,2,10,11-tetrahydroxyaporphine on both the OV-1 and OV-17 columns. Corydine, containing one TMS group, was eluted before the two tetrahydroxyaporphines on OV-1, but its retention time was greater than that of the tetrahydroxyisomers, 1,2,9,10- and 1,2,10,11-tetrahydroxyaporphine, on OV-17. The two tetrahydroxyisomers had similar retention times on OV-17, but the 1,2,9,10-isomer was eluted first on OV-1.

In contrast to the derivatized aporphines, the elution pattern of the silylated tetrahydroprotoberberines had a direct correlation with the number of silyl groups (or molecular weight) on OV-1 and an inverse correlation on OV-17. Three tetrahydroprotoberberines, viz. scoulerine, isocorypalmine, and stylopine, which yield 2,3,9,10-tetrahydroxyberbine on demethylation are eluted in increasing order of their molecular weight on OV-1 and in decreasing order of their molecular weight on OV-17, as is seen in Table 15.2. Similarly, coreximine is eluted before its demethylated product, 2,3,10,11-tetrahydroberbine, on OV-1. This pattern is reversed on OV-17. The two isomeric tetrahydroxyberbines, 2,3,9,10- and 2,3,10,11-tetrahydroxyberbine, are separated on OV-1 but have similar retention times on OV-17. The two naturally occurring isomeric alkaloids, coreximine and scoulerine, which are derived from reticuline in plants, are separated on OV-17 but have similar retention times on OV-1 at 260°C.

The separation of four derivatized aporphines and four tetrahydroberbines on OV-1 is shown in Figure 15.1. Although both classes of alkaloids have similar molecular weights (Table 15.2) the aporphines are all eluted before the tetrahydroberberines on a non-polar phase (OV-1), indicating that differences in the retention times of these alkaloids may be due to the geometry of the molecules.

A highly specific and sensitive method for the determination of berberine in urine was developed by Miyazaki et al.³ using chemical ionization fragmentography and (²H₃)berberine chloride as an internal standard. The berberine was reduced with sodium borohydride in methanol to give tetrahydroberberine, which was submitted to GC-MS analysis. Berberine concen-

TABLE 15.2

RETENTION TIMES OF SILYLATED DERIVATIVES OF APORPHINE AND TETRAHYDROPROTOBERBERINE ALKALOIDS²

Glass column: 6 ft by 4 mm, 3 % OV-1 or OV-17 on Gas Chrom Q 100-120 mesh at 260°C

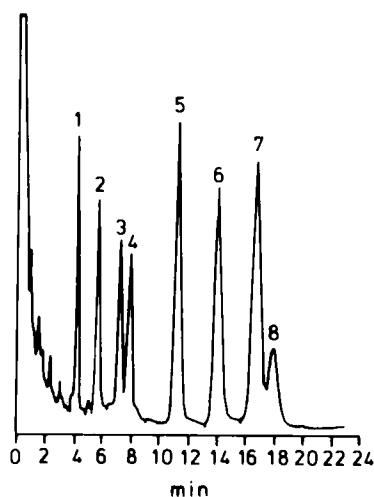
Compound	Mol.wt.	Number TMS groups	OV-1 t_R (min)	OV-17 t_R (min)
Aporphines				
Corydine (1-hydroxy-2,10,11-trimethoxy- aporphine)	413	1	4.9	7.2
Boldine (1,10-dimethoxy-2,9-dihydroxy- aporphine)	471	2	8.6	10.8
1,2,9,10-Tetrahydroxyaporphine	587	4	7.9	6.8
1,2,10,11-Tetrahydroxyaporphine	587	4	6.6	6.8
Tetrahydroprotoberberines				
Stylophine (2,3,9,10-bis(methylene- dioxy)-berbine)	323	0	9.2	25.0
Isocorypalmine (2-hydroxy-3,9,10-tri- methoxyberbine)	413	1	11.2	22.9
Scoulerine (2,9-dihydroxy-3,10-dimeth- oxyberbine)	471	2	13.6	19.1
Coreximine (2,11-dihydroxy-3,10-dimeth- oxyberbine)	471	2	13.8	20.5
2,3,9,10-Tetrahydroxyberbine	587	4	17.8	18.0
2,3,10,11-Tetrahydroxyberbine	587	4	16.6	18.2

FIGURE 15.1

CHROMATOGRAPHIC SEPARATION OF SILYLATED APORPHINES AND TETRAHYDROPROTOBERBERINES²

Glass column: 6 ft by 4 mm, 3 % OV-1 on Gas Chrom Q 100-120 mesh at 260°C

1 = Corydine, 2 = 1,2,10,11-Tetrahydroxyaporphine, 3 = 1,2,9,10-Tetrahydroxyaporphine,
 4 = Boldine, 5 = Isocorypalmine, 6 = Coreximine, 7 = 2,3,10,11-Tetrahydroxyberbine,
 8 = 2,3,9,10-Tetrahydroxyberbine



trations down to 1 ng/ml urine could be determined. Samples of 200 ml urine were used for the analysis. After addition of the internal standard (100 µg) the mixture was lyophilized and the residue extracted with ethanol. The solution was chromatographed over XAD-2, and the eluate, obtained with 1 M hydrochloric acid-methanol, evaporated to dryness - and the reduction carried out. The free base was liberated with potassium hydroxide and extracted with chloroform, the chloroform solution concentrated and analyzed. Recovery of berberine added to urine was $95.7 \pm 0.5 \%$.

Because N-n-propylnorapomorphine is reported to be several times more potent than apomorphine in several biological systems, Green et al.⁴ developed a GC-MS method for the detection, identification and quantification of aporphines. The O-trifluoroacetyl derivatives of a series of N-methyl- and N-propylaporphines as well as noraporphines were prepared and examined. The retention data are given in Table 15.3.

TABLE 15.3

RETENTION DATA OF APORPHINE DERIVATIVES⁴

The values are in methylene units. Column 183 cm by 2 mm, 1 % OV-17, temp.program. from 200°C

Compound	Trimethylsilyl derivatives	Trifluoroacetyl derivatives
Apomorphine	27.56	23.78
Apocodeine	28.50	26.62
Isoapocodeine	27.79	25.56
Bulbocapnine	31.60	31.00
2,10,11-Trihydroxy-N-methylaporphine	29.12	24.33
2,11-Dihydroxy-10-methoxy-N-methylaporphine	30.24	26.83
2,10-Dimethoxy-11-hydroxy-N-methylaporphine	30.88	29.33
N-n-Propylnorapomorphine	28.67	25.04
N-n-Propylnorapocodeine	29.52	27.67
10-Hydroxy-N-n-propylnoraporphine	29.08	25.80
11-Hydroxy-N-n-propylnoraporphine	27.07	24.33

TABLE 15.4

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF APORPHINE ALKALOIDS

Column	Solid support mesh	Stat.phase	%	Temperature	Comp. Prep.	Ref.
glass, 2 m x 5 mm I.D.	micro glass beads	SE-30	0.3	235°C	250°C alk.s.	1
glass, 6 ft x 4 mm	GQ 100-120	OV-1	3	260°C	alk.der.s.	2
glass, 1 m x 2 mm I.D.	GQ	OV-17	3	260°C	ber.qnt.ur.	3
glass, 163 cm x 2 mm I.D.	Supelcoport 100	Dex.300	1	230°C	pr alk.der.s.	4
		OV-17	1	from 200°C		

Abbreviations: Dex.300 = Dextsil 300, pr. = (temperature) programming, alk. = alkaloid, s. = separation, der. = derivative, ber. = berberine, qnt. = quantitative, ur = urine

15.2 REFERENCES

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- 2 J.L. Cashaw, K.D. McMurtey, L.R. Meyerson and V.E. Davis, *Anal. Biochem.*, 74 (1976) 343.
- 3 H. Miyazaki, E. Shirai, M. Ishibashi and K. Niizima, *J. Chromatogr.*, 152 (1978) 79.
- 4 J.F. Green, G.N. Jham, J.L. Neumeyer and P. Vouras, *J. Pharm. Sci.*, 69 (1980) 936.

Chapter 16

ISOQUINOLINE RELATED ALKALOIDS

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16.1 AMARYLLIDACEAE ALKALOIDS

Millington et al.¹ demonstrated the value of using gas chromatography-mass spectrometry for the analysis of a crude mixture of Amaryllidaceae alkaloids isolated from *Crinum glaucum*. Because of the low volatility of most Amaryllidaceae alkaloids they are unsuitable for direct analysis by gas chromatography-mass spectrometry. They were therefore converted to their trimethylsilyl derivatives, having the high volatility and stability required for gas chromatography and exhibiting mass spectra which provide useful structural information. The GC-MS data obtained with the TMS derivatives of some Amaryllidaceae alkaloids are listed in Table 16.1. The structure of some hydroxylic alkaloids and their TMS esters are shown in Figure 16.1

TABLE 16.1

GC-MS DATA OF THE TMS DERIVATIVES OF SOME AMARYLLIDACEAE ALKALOIDS¹

Compound	t _R (rel)	Principal m/e values (rel. intensities)
TMS-Ambelline (D)	1.00	403(100), 388(17), 373(19), 372(65), 282(56), 254(19), 241(69), 212(23), 211(64), 73(65),
TMS-Crinamine	0.83	373(82), 342(15), 252(28), 240(21), 224(30), 211(78), 181(100), 132(43), 73(40),
TMS-Criwelline	0.87	403(26), 373(39), 243(29), 212(19), 211(72), 185(16), 181(56), 162(18), 73(100), 70(34),
Bis-TMS-Deacetylbowdensine	1.28	463(49), 347(25), 305(14), 292(16), 244(12), 232(15), 231(35), 228(20), 204(19), 73(100),
Bis-TMS-Lycorine (C)	0.82	431(9), 340(8), 315(8), 250(9), 228(17), 227(100), 226(62), 75(9), 73(47),
Bis-TMS-Criglaucine (F)	1.31	463(59), 254(20), 232(49), 231(17), 218(23), 217(40), 204(13), 156(33), 147(20), 73(100),
Bis-TMS-Criglaucidine (D')	1.05	461(16), 319(10), 301(27), 211(19), 199(9), 181(24), 103(12), 75(13), 73(100), 70(13),
Unidentified (A)	0.64	343(54), 288(28), 287(16), 254(29), 227(24), 226(14), 201(18), 198(28), 171(16), 73(100),
Unidentified (B)	0.73	373(100), 372(21), 358(19), 284(18), 282(21), 256(30), 231(37), 218(18), 204(18), 73(58),
Unidentified (E)	1.20	403(100), 388(17), 373(26), 372(60), 282(56), 254(20), 242(19), 241(65), 211(64), 73(91),

FIGURE 16.1

ALKALOIDS FROM *CRINUM GLAUCUM*¹

I = Ambelline, II = criwelline, III = crinamine, IV = lycorine, V = deacetylbowdensine;
 R = H. Trimethylsilyl derivatives, R = TMS.

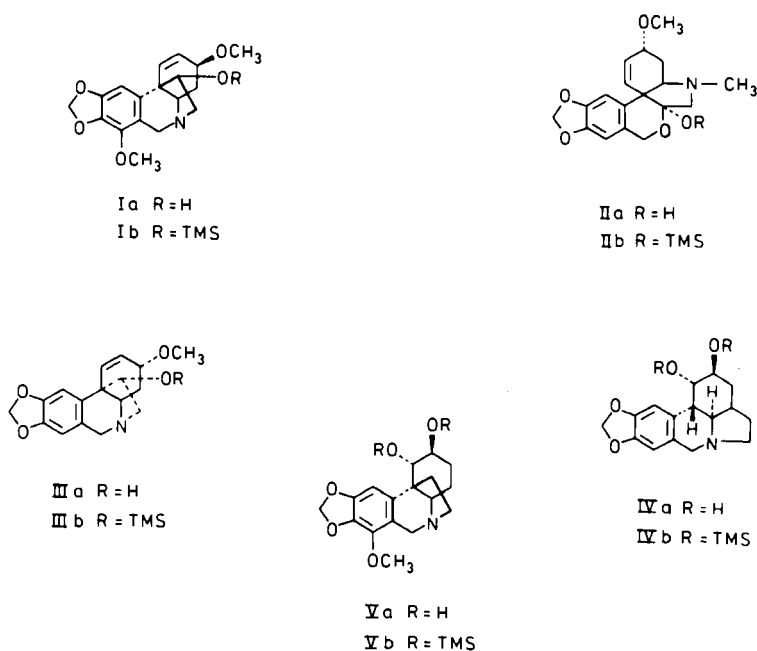
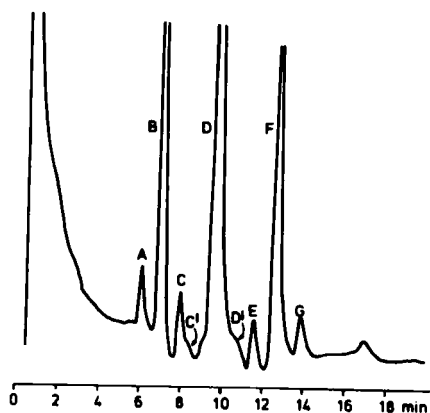


FIGURE 16.2

GAS CHROMATOGRAM OF *CRINUM GLAUCUM* ALKALOIDS AFTER TRIMETHYLSILYLATION¹

Column: 3 % OV-1 on Gas Chrom Q, temperature programming 215-250°C; for names see Table 16.1.



16.2 ERYTHRINA ALKALOIDS

To be able to carry out a comprehensive investigation of the alkaloid composition of a large and diverse collection of *Erythrina* species Millington et al.² applied gas chromatography-mass spectrometry as a tool for effecting complete characterization of the alkaloids in plant species or genus, of identifying new alkaloids and assigning structures to them, and of employing the alkaloidal composition established for individual species for a chemotaxonomic guide within the plant genus. Because most of the alkaloids encountered contained one or more hydroxyl groups, it was necessary to improve their volatility for the GC-MS analysis. This was achieved by conversion to their trimethylsilyl derivatives. The gas chromatography was carried out with a packed column of 3 % OV-17 on Gas Chrom Q. In order to identify the gas chromatographically separated compounds the gas chromatograph was interfaced with a low-resolution mass spectrometer. The alkaloids studied are shown in Figure 16.3.

FIGURE 16.3

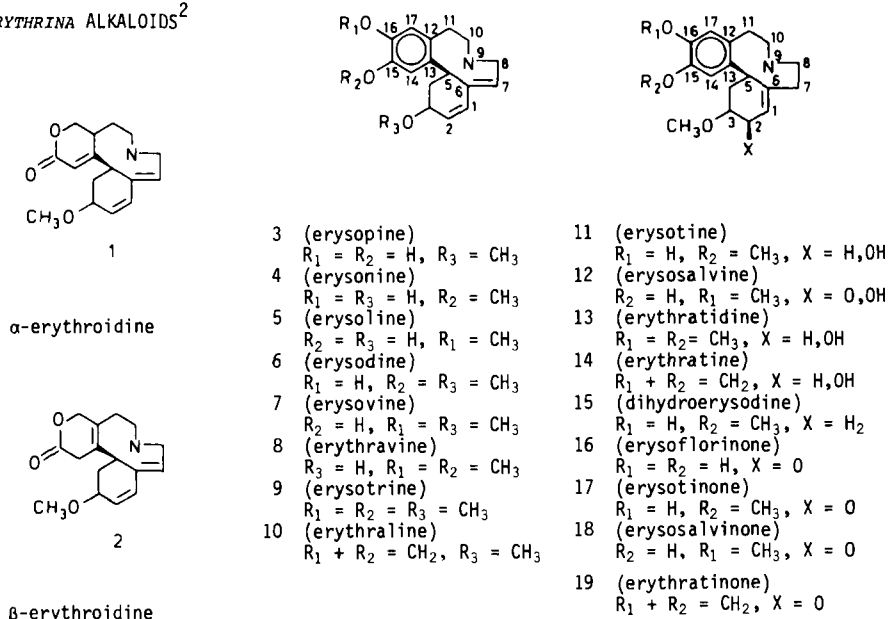
ERYTHRINA ALKALOIDS²

TABLE 16.2

RELATIVE RETENTION TIMES OF ERYTHRINA ALKALOIDS (FREE BASES AND TMS-DERIVATIVES)²

Column: glass, 6 ft by 2 mm I.D., 3 % OV-17 on Gas Chrom Q, temperature programming 225-250°C

Alkaloid	Relative retention time	
	Free base	TMS-derivative
β -Erythroidine	1.39	0.43
Erysotrine	1.17	
Erythraline	1.21	
Erysoline		0.95
Erysonine		0.89
Erysopine		0.95
Erythravine		1.07

TABLE 16.2 (continued)

Alkaloid	Relative retention time	
	Free base	TMS-derivative
Erysodine		1.00
Erysovine		1.07
Dihydroerysodine		0.93
Erysotine		1.18
Erysosalvine		1.25
Erythratidine		1.34
Erysoflorinone		1.58
Erysotinone		1.66
Erysosalvinone		1.47

TABLE 16.3

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF ISOQUINOLINE RELATED ALKALOIDS

Column	Solid support mesh	Stat.phase	%	Temperature	Comp.Preparation	Ref.
glass, 6 ft x 2 mm I.D. GQ	100-120	OV-1	3	215-250°C	pr. alk.der.s.	1
glass, 6 ft x 2 mm I.D. GQ		OV-17	3	225-250°C	pr. alk.der.s.	2

16.3 REFERENCES

- 1 D.S. Millington, D.E. Games and A.H. Jackson, *Proc.Int.Symp.Gaschromatogr.Massspectrom.*, 1972, 277.
- 2 D.S. Millington, D.H. Steinman and K.L. Rinehart, Jr., *J. Am. Chem. Soc.*, 96 (1974) 1909.

11.5 INDOLE ALKALOIDS

Chapter 17

TERPENOID INDOLE ALKALOIDS AND SIMPLE INDOLE ALKALOIDS

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17.1. TRYPTAMINE AND β -CARBOLINE ALKALOIDS

In a gas chromatographic study on biologically important amines, Fales and Pisano¹ investigated some indole bases, such as tryptamine and serotonin. When the indole bases were gas chromatographed on a 0.75 % SE-30 column on Gas Chrom P, extensive tailing was observed, probably due to adsorptive interactions between the compound and the support. However, when a thicker layer of SE-30 was used (4 %) the compounds showed well-formed peaks without tailing.

Holmstedt et al.² found that terminal primary amines condense readily with acetone, therefore a phenolic amine (serotonin) gives the corresponding trimethylsilyl ether-acetone condensation product when silylation is carried out in acetone solution. Primary amines may be entirely lost on a NGS column, but the acetone condensation products have a normal behaviour under these conditions. The relative retention times for indole bases related to tryptamine are summarized in Table 17.1.

TABLE 17.1

RELATIVE RETENTION TIMES FOR INDOLE BASES RELATED TO TRYPTAMINE²

Glass columns 6 ft by 4 mm with 7 % F-60 plus 1 % EGSS-Z at 182°C, and 10 % NGS at 216°C; Anthracene time 7.0 min and 6.6 min respectively - and 4.9 min on NGS at 227°C

Compound	F-60-Z, 182°C	NGS, 216°C
Anthracene	1.00	1.00
N,N-Dimethyltryptamine	1.05	1.68
N,N-Diethyltryptamine	1.71	2.14
4-Trimethylsilyloxy-N,N-dimethyltryptamine	2.89	-
5-Trimethylsilyloxy-N,N-dimethyltryptamine	3.19	3.21
6-Trimethylsilyloxy-N,N-dimethyltryptamine	3.70	3.74
7-Trimethylsilyloxy-N,N-dimethyltryptamine	2.23	1.72
5-Trimethylsilyloxy-N,N-diethyltryptamine	5.10	3.96
Tryptamine	1.00	-
Acetone condensation product of tryptamine	1.86	3.26
5-Methoxytryptamine	2.74	-
Acetone condensation product of 5-methoxytryptamine	4.50	9.50

TABLE 17.1 (continued)

Compound	F-60-Z, 182°C	NGS, 216°C
5-Methoxy-N,N-dimethyltryptamine	2.69	5.10
Serotonin	3.10	-
Acetone condensation product of serotonin	8.74	-
Trimethylsilyl ether of acetone condensations product of serotonin	5.29	-
		NGS, 227°C
4-Hydroxy-N,N-dimethyltryptamine	3.46	7.22
5-Hydroxy-N,N-dimethyltryptamine	5.77	15.0
6-Hydroxy-N,N-dimethyltryptamine	5.92	15.9
7-Hydroxy-N,N-dimethyltryptamine	4.41	11.9
5-Hydroxy-N,N-diethyltryptamine	8.11	

Holmstedt et al.² applied gas chromatography to solve the problem of the structure of the chief indole bases of *epená*, a South American snuff reported to produce hallucinations. With the gas chromatographic method mentioned above the following compounds were found: Tryptamine, N,N-dimethyltryptamine, 5-hydroxy-tryptamine, 4-hydroxy-N,N-dimethyltryptamine, 5-hydroxy-N,N-dimethyltryptamine, 6-hydroxy-N,N-dimethyltryptamine and 7-hydroxy-N,N-dimethyltryptamine.

Aguirell et al.³ investigated by means of gas chromatography certain species of *Virola* and other South American plants that have been used as sources of intoxicating snuffs by certain South American Indian tribes. The hallucinogen 5-methoxy-N,N-dimethyltryptamine and a number of other indoles were found. One Indian snuff proved to be unusually rich in alkaloids (11 %). Considerable differences in the alkaloid composition of different parts of single plants were encountered, N,N-dimethyltryptamine being the major component in the leaves and 5-methoxy-N,N-dimethyltryptamine in the bark of *Virola theidora*. Of the other species of *Virola* investigated, *V. rufula*, contained substantial amounts of tryptamines, whereas *V. multinervia* and *V. venosa* were almost devoid of alkaloids. *V. calophylla* contained high amounts of alkaloids only in the leaves. Two new β -carboline of a type carrying the substituents in the 6-position of the β -carboline nucleus were found in *V. theidora*, *V. rufula*, and *Anadenanthera (Piptadenia) peregrina*. From spectrometric and other data their structures were shown to be 2-methyl-6-methoxy-1,2,3,4-tetrahydro- β -carboline and 1,2-dimethyl-6-methoxy-1,2,3,4-tetrahydro- β -carboline. The alkaloids found are listed in Table 17.2 and their chemical structures in Figure 17.1. Typical gas chromatograms are given in Figure 17.2 and 17.3. The alkaloid bases were extracted with chloroform after basification of the methanolic extract, the salts back extracted into aqueous hydrochloric acid and the bases again extracted from the aqueous solution with chloroform after addition of sodium carbonate. The gas chromatographic separation was performed on packed columns of various polarities and the identification achieved by mass spectrometry.

TABLE 17.2

ALKALOIDS FOUND IN *VIROLA* AND OTHER SOUTH AMERICAN PLANTS³

N,N-Dimethyltryptamine	5-Hydroxy-N,N-dimethyltryptamine (bufotenine)
N-Methyltryptamine	5-Hydroxy-N-methyltryptamine
Tryptamine	5-Hydroxytryptamine (serotonin)
5-Methoxy-N,N-dimethyltryptamine	2-Methyl-1,2,3,4-tetrahydro- β -carboline
5-Methoxy-N-methyltryptamine	2-Methyl-6-methoxy-1,2,3,4,-tetrahydro- β -carboline
5-Methoxytryptamine	1,2-Dimethyl-6-methoxy-1,2,3,4,-tetrahydro- β -carboline

FIGURE 17.1

CHEMICAL STRUCTURES OF ALKALOIDS FOUND IN *VIROLA* AND OTHER SOUTH AMERICAN PLANTS³

1a Tryptamine, 1b N-methyltryptamine, 1c N,N-dimethyltryptamine, 2a 5-methoxytryptamine, 2b 5-methoxy-N-methyltryptamine, 2c 5-methoxy-N,N-dimethyltryptamine, 3a 5-hydroxytryptamine (serotonin), 3b 5-hydroxy-N-methyltryptamine, 3c 5-hydroxy-N,N-dimethyltryptamine (bufotenine), 4a 2-methyltetrahydro- β -carboline, 4b 2-methyl-6-methoxytetrahydro- β -carboline, 5 1,2-dimethyl-6-methoxytetrahydro- β -carboline, 6a tetrahydroharmine, 6b 2-methyltetrahydroharmine.

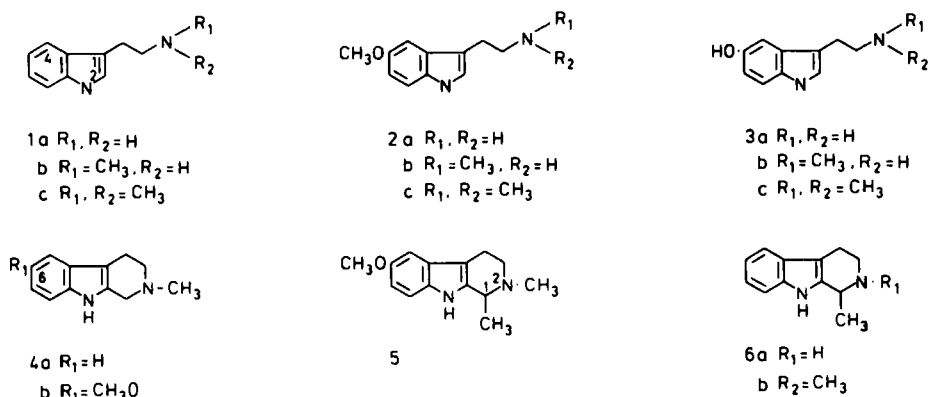


FIGURE 17.2

GAS CHROMATOGRAMS OF ALKALOID FRACTIONS OF SOUTH AMERICAN SNUFFS³

Column: 1.9 m by 3.2 mm I.D. packed glass column; 7 % F-60 + 2 % EGSS-Z on Gas Chrom P at 193°C. A: Extract of Epéna from Rio Cauaburi; B: Extract of Nyakwāna from Rio Tototobi. Upper chromatogram high magnification, lower chromatogram low magnification. A: 1 = DMT, 2 = MMT, 3 = MTHC, 4 = 5-MeO-DMT, 5 = 6-MeO-THC. B: 1 = DMT, 2 = MMT, 3 = 5-MeO-DMT, 4 = 5-MeO-MMT, 5 = 6-MeO-THC.

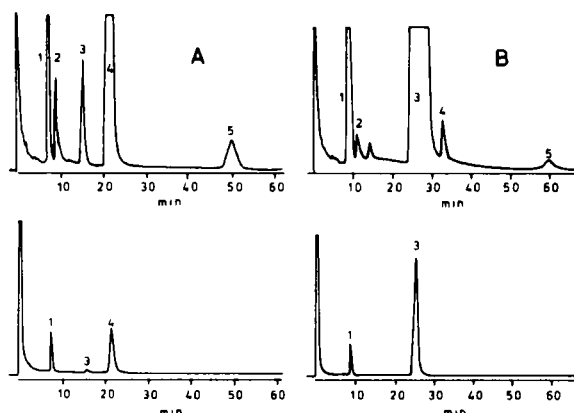
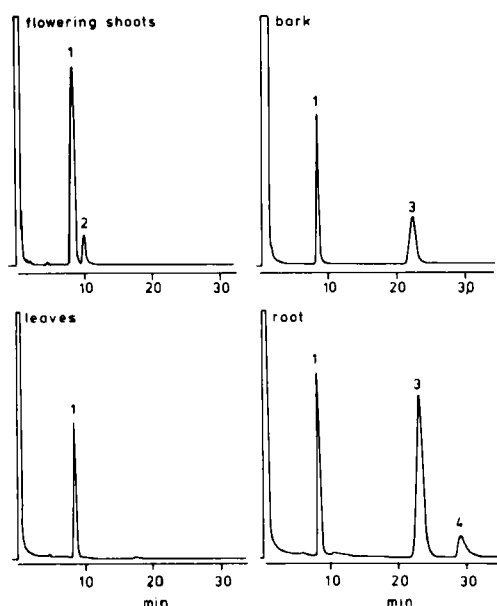


FIGURE 17.3

ALKALOIDS IN FLOWERING SHOOTS, LEAVES, BARK AND ROOTS OF *VIROLA THEIODORA*³

Glass column 1.8 m by 3.2 mm with 7 % F-60 + 2 % EGSS-Z on Gas Chrom P at 193°C
 1 = DMT, 2 = MMT, 3 = 5-MeO-DMT, 4 = 5-MeO-MMT.



Audette et al.⁴ performed a gas chromatographic screening of *Phalaris* species for alkaloids, mainly of the tryptamine type. Samples of 20 g dried plant material were extracted with ethanol in a Soxhlet apparatus, the ethanol was evaporated and the residue dissolved in dilute sulphuric acid. After addition of excess of ammonium hydroxide, the alkaloid bases were extracted with chloroform, and after concentration the gas chromatographic analysis was carried out on packed columns of different polarities, using Teflon tubings. After conditioning of the columns they were silanized with 10 μ l of Silyl 8 (Pierce Chem. Co.). The retention times of the alkaloids on the various columns used are given in Table 17.3.

17.2. HETEROYOHIMBINE ALKALOIDS

In a gas chromatographic study of the effect of methoxy substitution and configuration of heteroyohimbine alkaloids on the retention times on a 1 % SE-30 packed column, Beckett and Dwuma-Badu⁵ showed that they were in the order pseudo < allo < normal. The introduction of one methoxy group into the indole nucleus doubled the retention time, while two methoxy groups increased it by a factor of four. In Table 17.4 the retention times and the configurations of closed ring E heteroyohimbine alkaloids are listed.

TABLE 17.3

RETENTION TIMES OF TRYPTAMINE ALKALOIDS IN *PHALARIS* SPECIES⁴

Teflon tubing 6 ft by 1/8 inch I.D. packed with Amine 200, CHDMS and DEGS on Gas Chrom G (Amine 200 and CHDMS) and Chromosorb W (DEGS), at temperatures of 165°C, 195°C and 180°C, respectively.

Compound	Column		
	Amine 200	CHDMS	DEGS
Gramine	1.31	0.51	0.52
Hordenine	1.04	0.58	1.08
Dimethyltryptamine	12.08	3.03	3.29
5-Methyltryptamine	20.09	5.41	8.13
Tryptamine	18.35	4.28	6.58
N-Methyltryptamine	15.40	4.00	5.09
N,N-Dimethyl-5-methoxytryptamine	36.05	9.16	10.50
5-Methoxytryptamine	66.04	14.41	24.48
5-Methoxy-N-methyltryptamine	50.04	12.42	18.07
Bufotenine		42.16	74.00

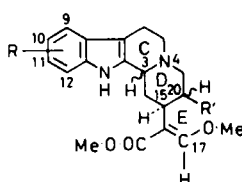
TABLE 17.4

RETENTION TIMES AND CONFIGURATIONS OF CLOSED RING E HETEROYOHIMBINE ALKALOIDS⁵

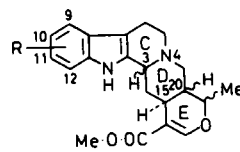
Glass column 1 m, packed with SE-30 1 % on Gas Chrom P, at 215°C

Alkaloid	Configuration R		Configuration of C-19-Me	Retention time (min)	pKa
Ajmalicine	Normal	H	α	10.5	6.31
Tetraphylline	Normal	11-OMe	α	22.5	6.39
Raunitorine	Normal	10-OMe	β	22.7	
Rauvanine	Normal	10,11-di-OMe	β	40.1	
Rauniticine	Allo	H	β	7.1	6.24
Tetrahydroalstonine	Allo	H	α	8.9	5.83
Raunitidine	Allo	11-OMe	β	16.3	6.20
Aricine	Allo	10-OMe	α	19.7	5.75
Reserpinine	Allo	11-OMe	α	20.3	6.01
Iso-reserpiline	Allo	10,11-di-OMe	α	34.0	6.07
Akuammigine	Epiallo	H	α	7.1	
Iso-reserpinine	Epiallo	11-OMe	α	15.6	6.49
Iso-raunitidine	Epiallo	11-OMe	β	17.9	6.42
Reserpiline	Epiallo	10,11-di-OMe	α	26.3	6.20
Iso-ajmalicine	Pseudo	H	α	5.3	
Mitrajavine	Pseudo	9-OMe	α	9.9	
Epirauvanine	Pseudo	10,11-di-OMe	β	23.6	

The structure of heteroyohimbine alkaloids with open ring E and closed ring E. In the alkaloids with open ring E (1) R = H or OCH₃ and R' = -C₂H₅ or -CH=CH₂, in the alkaloids with closed ring E (2) R = H, mono or di-OCH₃ groups.



I



II

17.3. *RAUWOLFIA* ALKALOIDS

17.3.1. Reserpine and rescinnamine

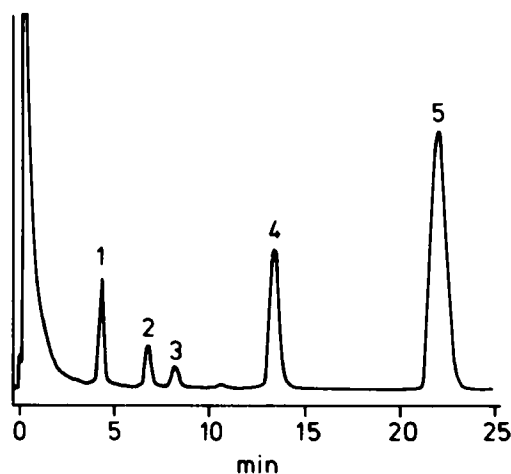
Settimj et al.⁶ described a gas chromatographic method for the estimation of reserpine and rescinnamine involving alkaline hydrolysis of the alkaloids and subsequent esterification of the acids formed by means of diazomethane. Reserpine gave quantitatively 3,4,5-trimethoxybenzoic acid methylester, whereas the *trans*-3,4,5-trimethoxycinnamic acid methylester, which should be expected for rescinnamine, was partly isomerized to the *cis*-trimethoxycinnamic acid methylester or formed an adduct with a molecule of methanol, yielding 3-methoxy-3-(3,4,5-trimethoxyphenyl) propionic acid methylester.

A gas chromatogram of the compounds are given in Figure 17.4

FIGURE 17.4

GAS CHROMATOGRAM OF HYDROLYSIS PRODUCTS OF RESERPINE AND RESCINNAMINE AFTER ESTERIFICATION⁶

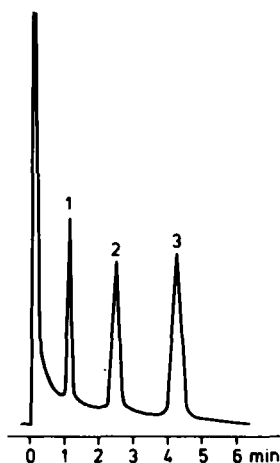
1 = methyl-3,4,5-trimethoxybenzoate, 2 = methyl-3-methoxy-3-(3,4,5-trimethoxyphenyl)propionate, 3 = *cis*-methyl-3,4,5-trimethoxycinnamate, 4 = *trans*-methyl-3,4,5-trimethoxycinnamate, 5 = the internal standard (methyl stearate). Column: glass, 2.40 m x 2 mm I.D., 15 % Apiezon L on Chromosorb W at 230°C.



Pantarotto et al.⁷ developed a gas chromatographic-mass spectrometric method for the identification and quantitative determination of reserpine in nanogram amounts in rat brain. The extraction of homogenized brain tissue was performed with acetone containing D₃-reserpine (as internal standard). After concentration of this extract it was used for gas chromatographic analysis on an OV-1 1% packed column on Gas Chrom Q at 285°C. No column adsorption, decomposition or C-3-epimerization was observed during the analysis. A comparison of the data obtained by means of gas chromatography-mass fragmentography with those obtained by radioisotopic assay gave satisfactory agreement. A gas chromatogram of the alkaloids is given in Figure 17.5.

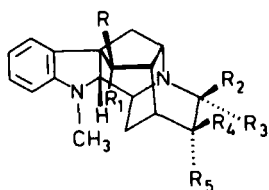
FIGURE 17.5

GAS CHROMATOGRAM OF DESERPIDINE (1), RESERPINE (2) AND RESCINNAMINE (3)⁷
 on a glass column, 40 cm by 4 mm I.D. packed with 1 % OV-1 on Gas Chrom Q at 285°C



17.3.2. Ajmaline

Forni⁸ described a method for quantitative determination of ajmaline in bark and root samples of *Rauwolfia vomitoria*. Because of the presence of many products in the raw methanolic extract of the crude drug, an extraction of the acidified extract was performed with chloroform prior to extraction of the alkaloids with the same solvent after adjustment of the pH to 8.5. Because of the polarity of the alkaloids, they were silanized before gas chromatography on a 3 % OV-17 on Chromosorb W column. A gas chromatogram showing the good separation of ajmaline from other alkaloids with similar structures and arbutin, which was used as an internal standard, is found in Figure 17.6.



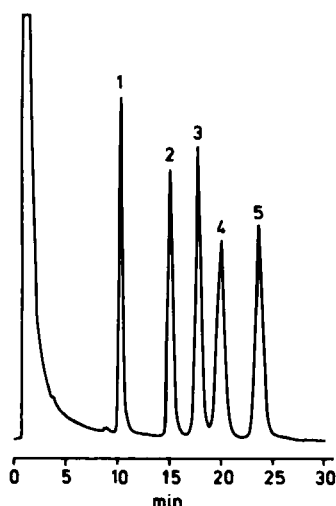
Ajmaline	R=R ₃ =OH	R ₁ =R ₂ =R ₅ =H	R ₄ = C ₂ H ₅
Isoajmaline	R=R ₂ =OH	R ₁ =R ₃ =R ₄ =H	R ₅ = C ₂ H ₅
Sandwichine	R ₁ =R ₃ =OH	R =R ₂ =R ₅ =H	R ₄ = C ₂ H ₅
Tetraphyllicine	R=OH	R ₃ =H	

FIGURE 17.6

GAS CHROMATOGRAM OF AJMALINE AND SOME RELATED ALKALOIDS⁸

on a glass column, 2 m by 3 mm I.D. packed with 3 % OV-17 on Chromosorb W at 270°C

1 = Arbutin (internal standard), 2 = sandwichine, 3 = isoajmaline, 4 = ajmaline, 5 = tetra-
phyllidine



Ten analyses of the same batch of crude drug were carried out. The results obtained were 2.41, 2.55, 2.52, 2.22, 2.41, 2.43, 2.36, 2.44 and 2.45 % of ajmaline with a mean of 2.43 %, a standard deviation of 0.098 % and a coefficient of variation of 4.05 %

17.4. *STRYCHNOS* ALKALOIDS

Of the *Strychnos* alkaloids, mainly strychnine and brucine have been examined by gas chromatography. The gas chromatography has mostly been carried out on non-polar or slightly polar stationary phases (SE-30, SE-52, XE-60, OV-1 and OV-17), but also more polar liquids have been employed (NGS, EGSS-Y, HI-EFF 8B). As stated by Brochmann-Hanssen and Fontan⁹ polar stationary phases are more selective than non-polar phases, but with increasing polarity of the stationary phase the retention times of the alkaloids are significantly increased. If brucine and less polar bases like vomicine and novacine are gas chromatographed on very polar stationary phases, their retention times will be very long. Such stationary phases are, therefore, not suitable for rapid analysis. Non-polar or slightly polar phases should be preferred for this type of alkaloids.

Strychnine and brucine in alkaloidal mixtures isolated from seeds of *Strychnos nux vomica* L. were separated on a SE-30 1.15 % column by Brochmann-Hanssen and Baerheim Svendsen¹⁰, whereas Bisset and Fouché¹¹ and Bisset, Dejestret and Fouché¹² preferred the slightly polar SE-52 for the examination of alkaloidal mixtures from *S. nux vomica* and *S. icaia*. The more polar alkaloids, such as diaboline and retuline, were eluted with short retention times at 230–280°C, while the less polar bases, like vomicine and novacine, had much longer retention

times. A typical gas chromatogram of some *Strychnos* alkaloids is given in Figure 17.7, the relative retention times of the same alkaloids are presented in Table 17.5 and their chemical formulas in Figure 17.8.

FIGURE 17.7

GAS CHROMATOGRAM OF SOME *STRYCHNOS* ALKALOIDS¹¹

on a stainless steel column, 6 ft by 1/8 inch packed with 5 % SE-52 on Aeropak 30

1 = Pseudostrychnine subsidiary peak (?), 2 = strychnine, along with peak 1 may indicate that pseudostrychnine is present as well, 3 = α -colubrine, 4 = β -colubrine, 5 = icajine, 6 = brucine, 7 = vomicine, 8 = novacine

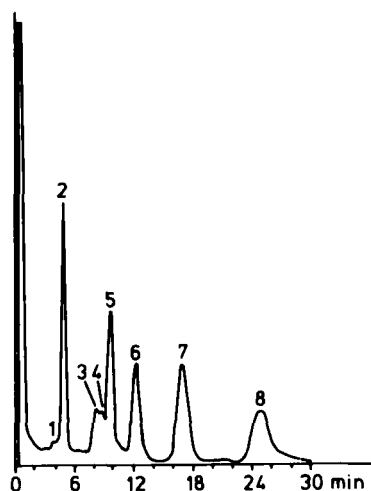


TABLE 17.5

RELATIVE RETENTION TIMES OF TERTIARY *STRYCHNOS* ALKALOIDS¹¹

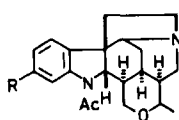
gas chromatographic conditions, see Figure 17.8

Column temperature	230°C	250°C		280°C	
	R_{stry}	R_{stry}	R_{bru}	R_{stry}	R_{bru}
Spermostrychnine	0.45	0.44	0.17		
Retuline	0.50	0.49	0.19		
Diaboline	0.76	0.69	0.26	0.69	0.31
Strychnospermine	0.82	0.75	0.29		
Strychnine *	1.00	1.00	0.38	1.00	0.45
Holstiine	1.03	0.94	0.36		
α -Colubrine		1.71	0.66	1.59	0.71
β -Colubrine		1.82	0.70	1.66	0.74
Icajine		1.96	0.78	1.88	0.84
Brucine **		2.61	1.00	2.24	1.00
Vomicine		3.62	1.41	3.10	1.39
Novacine		5.48	2.18	4.24	2.12

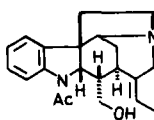
* Actual retention times: 230°C, 11.0; 250°C, 5.85; 280°C, 2.07 min.

** Actual retention times: 250°C, 15.2 and 280°C, 4.55 min.

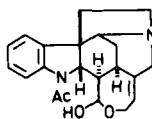
FIGURE 17.8

TERTIARY STRYCHNOS ALKALOIDS¹¹

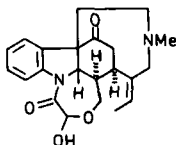
I



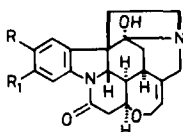
II



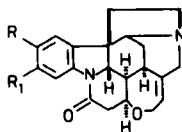
III



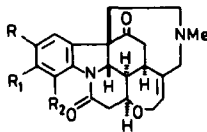
IV



V



VI



VII

I

R=H Spermotrychnine

R=OCH₃ Strychnospermine

II

Retuline

III

Diaboline

IV

Holstine

V

R=R₁=H PseudostrychnineR=R₁=OCH₃ Pseudobrucine

VI

R=R₁=H StrychnineR=H, R₁=OCH₃ α-ColubrineR=OCH₃, R₁=H β-ColubrineR=R₁=OCH₃ Brucine

VII

R=R₁=R₂=H IcajineR=R₁=H, R₂=OH VomisineR=R₁=OCH₃, R₂=H Novacine

Using gas chromatography of the N-oxides of the pseudo series of the alkaloids, decomposition of the alkaloids was observed in all cases, giving strychnine as the main peak on the chromatograms. The authors presumed that this decomposition might be due to the use of a stainless steel injector and column instead of working in an all-glass system.

Sondack and Koch¹³ carried out quantitative determinations of strychnine and brucine in pharmaceutical preparations using OV-1 and a glass column. Papaverine was used as an internal standard. The alkaloidal bases were extracted with chloroform after adding sodium hydroxide to the preparation. Hanks et al.¹⁴ utilized 1,3,5-triphenyl benzene as an internal standard for determinations of strychnine down to 2 μg in grain baits on an SE-30 5 % column.

Since plants may take up strychnine through the roots when used as baits above and below

ground to control rodents, Miller et al.¹⁵ developed a gas chromatographic method for determining strychnine residues in alfalfa. Samples of 50 g alfalfa were extracted with ethyl acetate after basification with sodium carbonate, the alkaloid back extracted into 0.1 N sulphuric acid and extracted with dichloromethane after basification again. After concentration the amount of strychnine was determined on a packed column with 1.5 % OV-17 as stationary phase. The average recovery of strychnine from spiked alfalfa (0.05 ppm) was 88 ± 11 % (8 determinations).

For the determination of strychnine in biological materials, Platonow et al.¹⁶ extracted the alkaloid from the biological material with chloroform as trichloroacetate. This strychnine salt is more soluble in chloroform than in water. The chloroform solution of the salt was injected into the gas chromatograph for analysis. Tissue concentrations of strychnine as low as 0.01 ppm could be detected. The recovery of strychnine added to liver was approximately, 90 %.

17.5. VARIOUS INDOLE ALKALOIDS

17.5.1. *vinca* alkaloids

The pharmacokinetics of vincamine after intravenous and oral application to the dog was investigated with gas chromatography by Laufen et al.¹⁷ Samples of plasma were extracted with dichloromethane:ethanol (99:1) after addition of quinine as internal standard and sodium hydroxide to pH 12.5. The residue obtained after evaporation of the solvents was treated with N-methyl-N-trimethylsilylfluoroacetamide and the reaction product taken up in hexane. Gas chromatography was performed on a 1 m long packed column with 1 % OV-1 or a 25 m long glass capillary column with OV-101. Good quantitative results were obtained. Detection limit was 0.5 ng/ml and the recovery of the extraction procedure about 85 %.

Hoppen et al.¹⁸ used a packed SE-30 column for the assay of vincamine in plasma. The molecular ion (m/e 426) and the parent peak (m/e 367) of the trimethylsilyl derivatives were assayed simultaneously by selected ion monitoring. Down to 30 pg/ml plasma could be assayed. The method is suitable for pharmacokinetic studies. Plasma samples of EDTA-anticoagulated blood (1-2 ml) were extracted with hexane after addition of tris(hydroxymethyl)aminomethane. The aqueous phase was frozen out and the hexane phase decanted into a conical glass tube. The solvent was evaporated and N-methyl-N-trimethylsilyltrifluoroacetamide was added. The reaction mixture was used for the gas chromatographic analysis on an 1 % SE-30 packed column on Chromosorb W HP at 210°C.

A number of closely related *vinca* alkaloids - such as stereo and structural isomers, ester homologues of vincaminic and apovincaminic acids - were separated on a packed OV-101 on Gas Chrom Q column by Gazdag et al.¹⁹. Derivatization (silylation with N,O-bis(trimethylsilyl)trifluoroacetamide) was needed for those alkaloids containing free hydroxy groups and/or a carboxylic acid group (vincaminic and apovincaminic acids). In Table 17.6 the retention times and elution temperatures of the *vinca* alkaloids investigated - as such and as derivatives - are given.

Polgár and Vereczkey²⁰ applied gas chromatography with a glass capillary column for the determination of apovincaminic acid, the main metabolite of apovincaminic acid ethylester (vinpocetine) in human plasma. Apovincaminic acid was recovered from plasma by addition of tetrabutylammonium hydroxide and extraction with chloroform. It was transformed into its

TABLE 17.6

RETENTION TIMES AND ELUTION TEMPERATURES OF VINCA ALKALOIDS²⁰

on a glass column, 1 m by 3.2 mm I.D. packed with 3 % OV-101 on Gas Chrom Q, with temperature programming from 200°C to 330°C, 5°C/min.

Compound	t _R (min)	Elution temperature
<i>cis</i> -Vincamenine	4.77	223.9
<i>cis</i> -Vincanol	4.77	223.9
<i>cis</i> -Isovincanol	4.77	223.9
<i>cis</i> -Vincamone	6.56	232.8
<i>cis</i> -Vincaminic acid	6.96	234.8
<i>cis</i> -Epivincamine	7.20	236.0
<i>trans</i> -Epivincamine	7.20	236.0
<i>cis</i> -Epivincaminic acid ethylester	7.44	237.2
<i>trans</i> -Vincamine	7.67	238.4
<i>trans</i> -Apovincaminic acid ethylester	7.78	238.9
<i>cis</i> -Vincamine	7.87	239.4
<i>cis</i> -Vincaminic acid ethylester	7.88	239.4
<i>cis</i> -Apovincamine	8.30	241.5
<i>trans</i> -Apovincamine	8.30	241.5
<i>cis</i> -Apovincaminic acid ethylester	8.88	244.4
<i>cis</i> -Apovincaminic acid	8.93	244.7
<i>cis</i> -11-Bromo-vincamine	10.98	254.9
<i>cis</i> -10-Bromo-vincamine	11.97	259.9
<i>cis</i> -Apovincaminic acid phenylester	14.92	274.6

methylester with diazomethane and gas chromatographed on an Sp 2100 glass capillary column, 10 m long by 0.25 mm I.D. at 220°C using 9-bromo-apovincaminic acid as an internal standard. With a NP-detector the detection limit was 2 ng/ml plasma.

17.5.2. *Physostigma* alkaloids

Physostigmine salicylate in 0.5 % aqueous solution was determined by Teare and Borst²¹ by freeze-drying of samples of 0.2 ml, and conversion of the compound into its trimethylsilyl derivative by dissolving the residue in 5 µl of dry pyridine and 10 µl of N,O-bis(trimethylsilyl)acetamide. The solution was allowed to stand for 1 h before injecting 1.2 µl into the gas chromatograph. A 3.8 % SE-30 on Diatoport S column and a temperature of 145°C was used for the analysis. The physostigmine TMS and the salicylic acid TMS derivatives were eluted separately. Routine analysis over several days gave a precision of ± 11.5 %.

17.5.3. *Aspidosperma* alkaloids

The usefulness of the application of a directly coupled gas chromatograph-mass spectrometer for the analysis of complex alkaloid mixtures was demonstrated by Thomas et al.²² in an investigation of the alkaloids in *Aspidosperma neblinae*. A mixture of the minor alkaloids was first chromatographed on alumina columns and the fractions obtained were gas chromatographed on a packed column, 1 % SE-30 on Gas Chrom Q, by temperature programming (150-250°C). The excellent resolution of the gas chromatograph and the mass spectra obtained led to the identification of 11 alkaloids. The alkaloids and the percentages in which they were found in the extract are given in Table 17.7. A gas chromatogram of some alkaloids is given in Figure 17.9.

TABLE 17.7

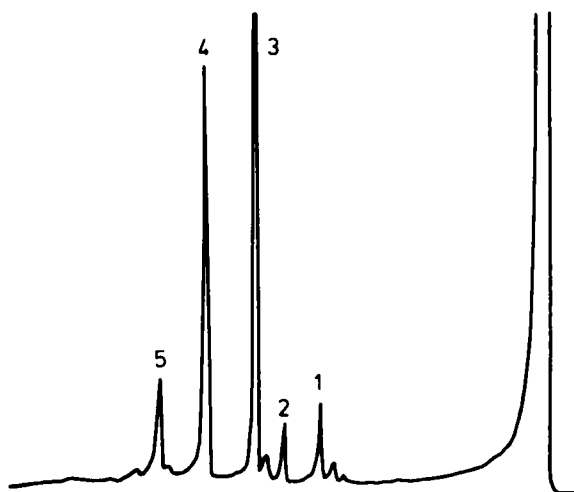
ALKALOIDS OF *ASPIDOSPERMA NEBLINAE*²²

	% of extract	peak no.
Aspidospermidine	0.1	
1,2-Dehydroaspidospermidine		
Deacetylpyrifolidine	3.3	1
1,2-Dehydrodeacetylpyrifolidine		
Demethoxyaspidospermine	0.9	
Aspidospermine	1.3	2
Demethylaspidospermine	4.7	
Pyrifolidine	26.3	3
Aspidocarpine	18.7	4
Eburnamonine	0.05	
Neblineine	0.8	5

FIGURE 17.9

GAS CHROMATOGRAM OF ALKALOIDS IN *ASPIDOSPERMA NEBLINAE*²²

on a glass column, 5 ft by 1/8 inch packed with 1 % SE-30 on Gas Chrom Q, with temperature programming from 150°C to 250°C. For numbering of peaks, see Table 17.6.



During a detailed investigation of the alkaloids of *Voacanga africana* Stapf, by Thomas and Bieman²³ a number of alkaloids were isolated by means of gas chromatography. They were identified by comparison of their mass spectra with available data. However, only few data of the gas chromatographic experimental conditions were given in the paper.

17.5.4. *Uncaria* alkaloids

Phillipson and Hemingway²⁴ applied a combination of thin-layer chromatography, gas-liquid chromatography, ultraviolet spectroscopy and mass spectrometry techniques for the alkaloid screening of herbarium samples of the genus *Uncaria* (Rubiaceae). Some sixty alkaloids were distinguished by the screening procedure, and they represented heteroyohimbine, oxindole, roxburghine, simple β -carboline, pyridine-indole-quinolizidine and gambirtannine types. Gas chromatograms of some alkaloids included in the screening are shown in Figure 17.10 and the retention times of some of the *Uncaria* alkaloids are listed in Table 17.8.

FIGURE 17.10

GAS CHROMATOGRAM OF SOME *UNCARIA* ALKALOIDS²⁴

on a glass column, 2 ft by 1/4 inch I.D., packed with 5 % SE-52 on Varaport 30, at 240°C. For numbering of peaks, see Table 17.7.

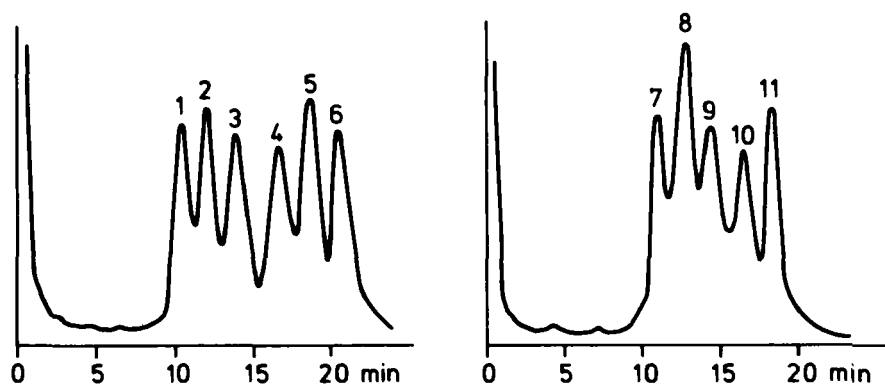


TABLE 17.8

RETENTION TIMES OF *UNCARIA* ALKALOIDS²⁴

For experimental conditions, see Figure 17.10.

Alkaloid	t_R (min)	No.	Alkaloid	t_R (min)	No.
<i>Pentacyclic heteroyohimbines</i>					
Ajmalicine	20.6	6	Rauneticine	15.5	
Isoajmalicine	10.7	1	<i>Tetracyclic heteroyohimbines</i>		
Mitrajavine	19.5		Dihydrocorynantheine	18.8	5
Tetrahydroalstonine	17.5		Gambirine	-	
Akuammigine	14.3	3	Speciogynine	37.7	
4-R akuammigine N-oxide	14.3		Hirsutine	12.2	2
19- <i>epi</i> -Ajmalicine	19.5		Hirsuteine	11.8	
3-Iso-19- <i>epi</i> -ajmalicine	12.7				

CHEMICAL STRUCTURES OF *UNCARIA* ALKALOIDS²⁴

- I Pentacyclic heteroyohimbine (R = H, OH or OMe)
 II Tetracyclic heteroyohimbine (R = H, OH or OMe, R' = Et or vinyl)
 III Pentacyclic oxindole (R = H, OH or OMe)
 IV Tetracyclic oxindole (R = H, OH or OMe, R' = Et or vinyl)

I-IV can exist as isomers defined as

	C-3 H	C-20 H
allo	α	α
epiallo	β	α
normal	α	β
pseudo	β	β

V Roxburghine C	C-3 H	C-19 H
- D	α	α
- E	β	α
	β	β

- VI Harmane (R = H)
 Harmine (R = OMe)

III and IV can exist as A or B isomers in which the lactam carbonyl can lie below (A) or above (B) the plane of the C-D rings

VII Harmaline

- VIII Angustine (R = CH=CH₂, R' = H)
 Angustoline (R = CH(OH)Me, R' = H)
 Angustidine (R = H, R' = Me)

- IX Gambirtannine (R = H₂)
 Oxogambirtannine (R = O)
 X Dihydrogambirtannine

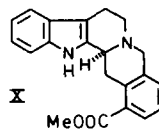
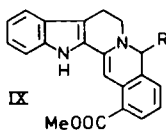
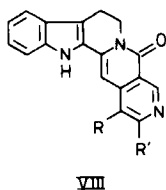
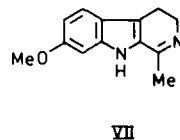
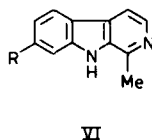
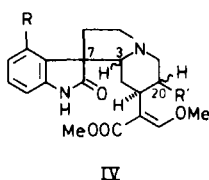
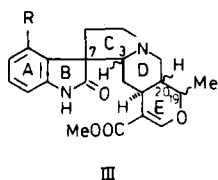
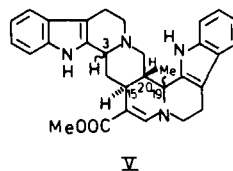
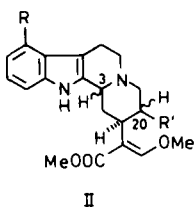
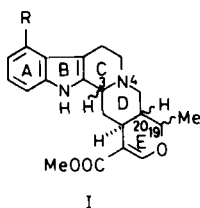


TABLE 17.8 (continued)

Alkaloid	t _R (min)	No.	Alkaloid	t _R (min)	No.
Tetracyclic heteroyohimbines (continued)			Tetracyclic oxindoles (continued)		
Mitraciliatine	22.3	4	Isocorynoxine	10.3	10
Corynantheidine	16.9		Rotundifoline	16.0	
Mitragynine	33.2		Rhynchociline	17.9	
Isocorynantheidine	16.7		Rhynchophylline	10.9	
Speciociliatine	32.1		Rhynchophylline N-oxide	10.9	7
			Corynoxine	10.3	
			Isorotundifoline	16.0	
			Ciliaphylline	17.9	11
			Corynoxine	10.2	
			Corynoxine B	10.2	
			Speciofoline	15.5	
Pentacyclic oxindoles					
Isomitraphylline	14.0	9			
Isomitraphylline N-oxide	14.0				
Javaphylline	23.2				
Mitraphylline	14.0				
Mitraphylline N-oxide	14.0	8			
Isopteropodine	12.4		Roxburghine C	-	
Isopteropodine N-oxide	12.4		Roxburghine D	-	
Pteropodine	12.4		Roxburghine E	-	
Pteropodine N-oxide	12.4		Dimeric indole alkaloid	-	
Speciophylline	12.4				
Speciophylline N-oxide	12.4		Harmine	0.6	
Uncarine F	12.4		Harmine	1.5	
Uncarine F-oxide	12.4		Harmaline	1.4	
Uncarine A	13.5				
Uncarine B	13.5		Angustine	-	
			Angustidine	-	
			Angustoline	-	
Tetracyclic oxindoles					
Isorhynchophylline	10.9		Gambirtannine	-	
anti-Isorhynchophylline-			Dihydrogambirtannine	20.8	
N-oxide	10.9		Oxogambirtannine	-	

TABLE 17.9

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF TERPENOID INDOLE ALKALOIDS AND SIMPLE INDOLE ALKALOIDS

Column	Solid support mesh	Stat.phase	%	Temperature	Comp. Prep.	Ref.
glass S, 6 ft	GP AWS 80-100	SE-30	0.75	160°C	ind.b.	1
glass, 6 ft x 4 mm	GP S 80-100	SE-30	4	205°C		
-	-	F-60	7	182°C	ind.b.pm.	2
-	-	+ EGSS-Z	1	216°C		
glass, 1.8 m x 3.2 mm	GP AWS 100-120	NGS	10	216°C	alk.id.MS.	3
-	-	F-60	7	193°C		
- 2.25 m x 3.2 mm	-	+ EGSS-Z	2	193°C		
-	-	SE-30	5	180°C		
Teflon, 6 ft x 1/8 in	I.D. CW AWS 80-100	OV-17	5	180°C	alk.id.	4
-	-	DEGS	1	180°C		
-	CG AWS 60-80	Amine 220	0.5	165°C	alk.s.	5
-	CG 60-80	CHDMS	0.4	195°C		
1 m	GP AWS 60-80	SE-30	1	215°C	rs.rc.qnt.	6
glass, 2.4 m x 2 mm I.D.	CW AW 80-100	Apiezon L	15	230°C		
glass, 40 cm x 4 mm I.D.	GQ 100-120	OV-1	1	285°C	rs.qnt.br.	7
glass, 2 m x 3 mm I.D.	CW HP 100-120	OV-17	3	270°C		
glass, 3 ft x 0.07 in I.D.	GP AWS 80-100	SE-30	1	225°C	aj.qnt.pm.	8
-	-	XE-60	1	220°C		
-	-	EFSS-Y	1	230°C		
-	-	HI-EFF 8B	1	240°C		

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Chapter 18

ERGOT ALKALOIDS

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18.1 LYSERGIC ACID DIETHYLAMIDE - LSD

The earliest investigations on gas chromatography of Ergot alkaloids were performed with lysergic acid diethylamide, LSD, in order to develop methods for its detection and identification in trace amounts in narcotic seizures; i.e. in sugar cubes impregnated with the psychomimetic drug. Due to the low volatility of the compound, Radecka and Nigam¹ hydrogenated it prior to gas chromatography to increase its stability. LSD was extracted from the sugar cubes as a free base with dichloromethane, hydrogenated with Adam's catalyst and gas chromatographed on 0.2 % SE-30 on micro glass beads. The hydrogenated LSD was eluted in 3.6 min at a column temperature of 240°C. By means of a stream splitter, eluates were collected and analyzed by thin layer chromatography for confirmation of identity. Convincing thin-layer chromatograms were obtained.

Because of the low sensitivity and non-reproducibility of the method developed by Radecka and Nigam¹ - 50 µg failed to give convincing detectable results - Katz et al.² developed a method for direct gas chromatography of LSD in submicrogram amounts using a packed column of SE-30, 0.3 %, on glass micro beads. Symmetrical peaks were obtained that were well suited for quantitative work. The detector response was linear at low concentrations and 0.5 µg of LSD could easily be detected and quantified.

Lerner³ developed a GLC method for detection of LSD directly on a 2 % SE-52 column at 250°C in an all-glass system, which minimize decomposition. However, for greater sensitivity and stability in gas chromatography, the author preferred to use the trimethylsilyl derivative of LSD. N,O-bis(trimethylsilyl)acetamide was utilized as silylation reagent and LSD was solved in dimethylformamide by the reaction, which was performed at 55°C for 30 minutes. The trimethylsilyl derivative of LSD was gas chromatographed at 245°C - also on a 2 % SE-52 column. LSD was extracted from sugar cubes with chloroform-methanol (9:1). The extraction was performed from aqueous solution after addition of bicarbonate. Figure 18.1 shows a gas chromatogram of 30 µg LSD and Figure 18.2 one obtained with 0.2 µg of the trimethylsilyl derivative of LSD.

Bailey et al.⁴ synthesized a series of dialkylamides of lysergic and isolysergic acid in order to distinguish LSD from these compounds. The following compounds were made:

- I Lysergic acid dimethylamide
- II Isolysergic acid dimethylamide
- III Lysergic acid diethylamide
- IV Isolysergic acid diethylamide
- V Lysergic acid methylpropylamide
- VI Isolysergic acid methylpropylamide
- VII Lysergic acid ethylpropylamide
- VIII Isolysergic acid ethylpropylamide
- IX Lysergic acid dipropylamide
- X Isolysergic acid dipropylamide

FIGURE 18.1

GAS CHROMATOGRAM OF 30 μg LSD³

on a stainless steel column, 1.5 m by 3 mm I.D., packed with glass micro beads, coated with 0.2 % SE-30 at 270°C

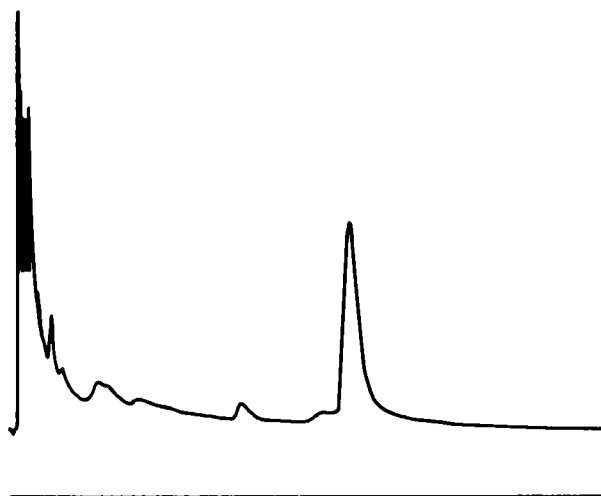
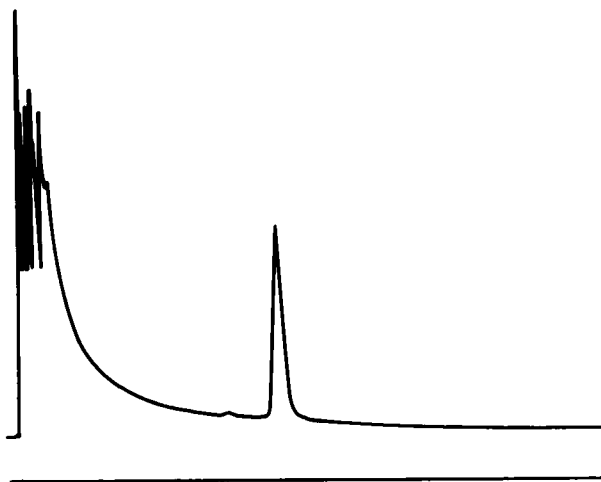


FIGURE 18.2

GAS CHROMATOGRAM OF 0.2 μg TRIMETHYLSILYL DERIVATIVE OF LSD

Gas chromatographic conditions, see Figure 18.1



The compounds were gas chromatographed on a 3 % SE-30 column as pure compounds at 225°C and as silylated compounds at 250°C, as shown in Table 18.1.

TABLE 18.1

RETENTION TIMES (MINUTES) OF LYSERGIC AND ISOLYSERGIC ACID AMIDES⁴
on a 3 ft long glass column, packed with 3 % SE-30 on Chromosorb W

Compound *)	3 % SE-30 225°	3 % SE-30 250° silylated compound
I/II	20.0	11.1
III/IV	24.7	13.6
V/VI	28.9	15.2
VII/VIII	32.3	**)
IX/X	35.6	19.4

*) For names of compounds see text, **) this compound was completely decomposed

As can be seen it is easy to distinguish LSD from its homologues and from the isomeric pair V and VI by using SE-30. Retention times increase with molecular weight, and the isomeric pairs are not separated under these conditions. Some decomposition of the amides and their silylated derivatives took place in the gas chromatographic process.

Lerner and Katsiaficas⁵ studied the separation of a number of hallucinogenic drugs: N,N-dimethyltryptamine, mescaline, psilocybine, ibogaine and lysergic acid diethylamide by gas chromatography on a 2 % SE-52 column. Only ibogaine gave a good, sharp gas chromatographic peak, and one microgram could be detected. The other compounds gave either asymmetrical peaks or relatively poor sensitivity. They were, therefore, converted into trimethylsilyl derivatives using dimethylformamide, N,O-bis(trimethylsilyl)acetamide and 4,5,6-trimethylchlorosilane (1:4:1) as solvent-reaction mixture and heating for one hour at 70°C. When ibogaine was present in a complex mixture, less than 50 % was usually converted to the trimethylsilyl derivative, but converted and unconverted ibogaine appeared as an easily recognizable double peak on the gas chromatogram. The results are presented in Table 18.2.

TABLE 18.2

GAS CHROMATOGRAPHY OF LSD AND OTHER HALLUCINOGENIC DRUGS⁵
on a 2 % SE-52 column

Compound	Temperature °C		t _R (min)
	Column	Injection port	
Dimethyltryptamine	140	190	8.0
Dimethyltryptamine (TMS derivative)	140	190	10.0
Dimethyltryptamine (TMS derivative)	Programmed	260	12.0
Mescaline	140	190	5.4
Mescaline (TMS derivative)	140	190	32.8
Mescaline (TMS derivative)	Programmed	260	19.7
Psilocybine	180	235	4.6
Psilocybine (TMS derivative)	180	235	21.8
Psilocybine (TMS derivative)	Programmed	260	30.6
Ibogaine	220	280	11.7
Ibogaine (TMS derivative)	220	280	12.7
Ibogaine	Programmed	260	39.3
Ibogaine (TMS derivative)	Programmed	260	39.9
Lysergic acid diethylamide	250	280	14.6
Lysergic acid diethylamide (TMS der.)	245	260	11.8
Lysergic acid diethylamide (TMS der.)	Programmed	260	47.6

To characterize LSD in illicit preparations, Jane and Wheals⁶ applied on-column silylation of LSD. N,O-bis(trimethylsilyl)acetamide was found to give better results than N-methyl-N-trimethylsilyl-trifluoroacetamide as silylating reagent, the latter resulted in lower yields of LSD and led to greater variation in the peak heights of replicated injections. The use of glass columns was found to be essential if on-column decomposition was to be avoided. The on-column silylation procedure was found to work very well, and linear, reproducible graphs could be obtained for solutions containing from 0.1 to 5 µg of LSD.

Sperling⁷ isolated LSD from different LSD tablets by column chromatography, converted it into its trimethylsilyl derivative by N,O-bis(trimethylsilyl)trifluoroacetamide and heating for one hour at 80°C. He was able to separate LSD-TMS from the N-methyl-N-propyl-isomer TMS. The LSD free base has a retention time that is only slightly longer than that of the TMS derivative and so, if the silylation is incomplete, LSD will appear as a shoulder on the main peak on the 0.25 % OV-17 column, using micro glass beads as solid support. Such columns do not have a very long life time because of bleeding off of the stationary phase at the temperature used.

18.2 ERGOT ALKALOIDS

The Ergot alkaloids may be divided into two groups. One group comprises a series of simpler ergoline derivatives, the clavine alkaloids, the other group may be considered as amides of lysergic acid. Whereas the first group of alkaloids has low molecular weights (mol.wt. 238-260, fumigaclavine A 229) the amide type of alkaloids have such high molecular weights that only the simpler amides such as lysergic acid amide, lysergic acid diethylamide (mol. wt. 323) and ergometrine pass a gas chromatographic column.

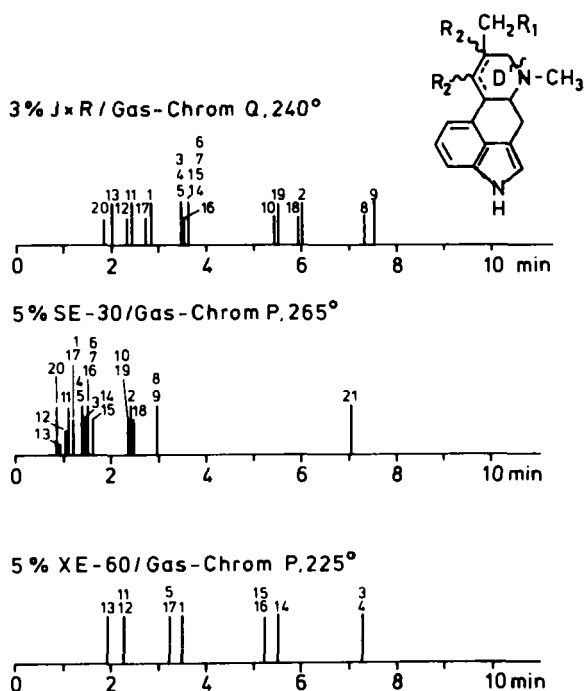
Agurell and Ohlsson⁸ gas chromatographed a number of clavine alkaloids on three different stationary phases, JxR 3 %, SE-30 5 % and XE-60 5 %. All clavine alkaloids could be chromatographed with satisfactory results on JxR and SE-30 columns and most satisfactorily on XE-60. The alkaloids containing hydroxyl groups showed limited tailing. Attempts to overcome this by formation of suitable derivatives (trimethylsilyl, heptafluorobutyl or trifluoroacetyl) yielded no adequate results with extracted alkaloid mixtures. None of the columns were able to separate stereoisomers. In Figure 18.3 the separation of the clavine alkaloids inclusive LSD is given on the columns utilized. Of the lysergic acid derivatives only LSD, lysergic acid amide and lysergic acid methylcarbinolamide - the last one after pyrolysis in the injector to lysergic acid amide - could be chromatographed on SE-30 and JxR columns at comparatively high temperatures. Thus, lysergic acid amide had a retention time of 14.7 minutes on 3 % JxR at 240°C. Sondack⁹ developed a method for the gas chromatographic determination of ergometrine as trimethylsilyl derivative. As silylating reagent, a mixture of 75 µl of N-trimethylsilyldiethylamine, 150 µl of dry pyridine and 100 µl of N-trimethylsilylimidazole was used. Brucine was utilized as an internal standard and quantitative estimations carried out for ergometrine maleate in tablets and injectables, containing 0.2 mg per tablet or milliliter. A residual standard deviation of ± 1 % for injectables and ± 3 % for tablets was found, and a relative error of less than 1 % for injectables. Degraded samples also showed on the chromatograms peaks of derivatized ergometrine and derivatized lumi-ergometrine.

A method for the gas chromatographic determination of agroclavine was developed by Barrow and Quigley¹⁰. The trifluoroacetyl derivative and the trimethylsilyl derivative were chroma-

FIGURE 18.3

RETENTION TIMES OF CLAVINE ALKALOIDS⁸

on 3 % JxR at 240°C, 5 % SE-30 at 265°C and 5 % XE-60 at 225°C (6 ft by 3 mm glass columns). 1 = agroclavine, 2 = elymoclavine, 3 = chanoclavine-I, 4 = chanoclavine-II, 5 = isochanoclavine-I, 6 = setoclavine, 7 = isosetoclavine, 8 = penniclavine, 9 = isopenniclavine, 10 = α -dihydrolysergol, 11 = festuclavine, 12 = pyroclavine, 13 = costaclavine, 14 = fumigaclavine B, 15 = fumigaclavine A, 16 = lysergene, 17 = lysergine, 18 = lysergole, 19 = isolysergole, 20 = cycloclavine, 21 = LSD.



tographed. Derivatization led to increase of the thermostability and change in retention times, permitting a better GLC identification. When using 1-3 % of SE-30, an extensive tailing of TMS-agroclavine took place, presumably by partial adsorption to active sites in the solid support. With a higher percentage of stationary phase, no tendency to tailing was observed. The OV-phases showed very long retention times and offered no advantage over the SE-phases. The TMS-agroclavine gave one single peak on the gas chromatograms, whereas the TFA-agroclavine gave two peaks of derivatized agroclavine and sometimes, also, a peak of underivatized agroclavine. The authors were not able to reproduce a single product or reproducibly obtain a consistent mixture of products with trifluoroacetic anhydride.

Because of the low volatility and thermal instability at high temperatures of the dihydroergotoxine alkaloids, Szepesi and Gazdag¹¹ developed a gas chromatographic method for the separation and identification of these alkaloids (dihydroergocristine, dihydroergokryptine

and dihydroergocornine) based on quantitative decomposition of the alkaloids catalyzed by the metal surface of the injection port and different migration rates of the peptide moieties from the various alkaloids during the decomposition. The free bases were injected for gas chromatography - and, depending on the temperature of the injection port, the chromatogram varied, as can be seen in Figure 18.4.

FIGURE 18.4

GAS CHROMATOGRAM OF DIHYDROERGOTOXINE ALKALOIDS¹¹

on a stainless steel column, 1 m long, packed with 2 % Dexsil 300; temperature programming 180-280°C at 5°C/min; injection port temperature (A) 195°C, (B) 210°C, (C) 235°C, (D) 290°C
1 = dihydroergocornine, 2 = dihydroergokryptine, 3 = dihydroergocristine, x = unknown.

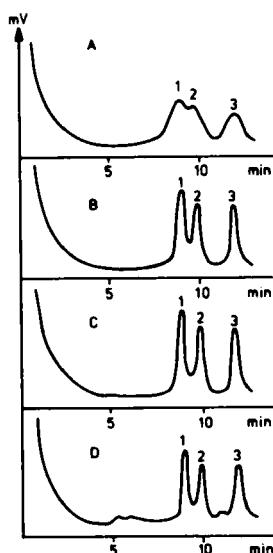


TABLE 18.3

RETENTION TIMES OF ERGOTOXINE AND DIHYDROERGOTOXINE ALKALOIDS¹¹

on the columns as described in Figure 18.4; temperature: 200°C for 18 min, then programmed from 200°C to 300°C at 20°C/min; temperature injection port: 235°C.

Compound	t_R (min)	Column temp. °C	Compound	t_R (min)	Column temp. °C
Ergocornine	9.01	225	Ergocristine	14.6	253
Ergocorninine			Ergocristinine		
Dihydroergocornine			Dihydroergocristine		
Dihydroergocorninine			Dihydroergocristinine		
Ergokryptine	10.03	230	Phenylbutazone	11.92	239
Ergokryptinine					
Dihydroergokryptine					
Dihydroergokryptinine					

De Zeeuw et al.¹² developed a method for the identification of ergotamine present in putrified blood samples. The samples were hydrolyzed with hydrochloric acid, the pH adjusted to 9.5 and extraction performed with chloroform. Gas chromatography of the residue obtained in this way on a SE-30 column gave one major peak. By computerized GC-MS, the compound responsible for the peak was identified as a cyclic dipeptide, phenylalanine-proline lactam. A minor peak was identified as its pyruvoyl precursor. In putrified blood the L-phenylalanine-L-proline lactam dominated quantitatively, but also small amounts of L-phenylalanine-D-proline lactam were found. In a hydrolysate of ergotamine in water and non-decomposed blood, L-phenylalanine-D-proline lactam was the major component, whereas L-phenylalanine-L-proline lactam was present in minor amounts together with its pyruvoyl precursor. The presence in blood samples of these compounds seems to be specific for ergotamine, dihydroergotamine and their respective -inine stereoisomers.

In a later publication, Van Mansvelt et al.¹³ stated that the degradation of ergotamine took place only to a limited extent during the acid hydrolysis and that it occurred mainly in the injection port of the gas chromatograph at the high temperatures used. They studied the applicability of this thermal decomposition of six ergot-peptide alkaloids: ergotamine, ergosine, ergocristine, ergokryptine, ergocornine and ergostine in connection with their identification with gas chromatography. The gas chromatography was performed in an all-glass system and the injection port temperature was 300°C. The decomposition pathways of ergotamine are given in Figure 18.5 and a gas chromatogram of the degradation products in Figure 18.6.

FIGURE 18.5

DECOMPOSITION PATHWAYS OF ERGOTAMINE¹³

1 = Ergotamine. LSA indicates the lysergic acid moiety. The dashed line indicates that the cleavage takes place between the α -nitrogen atom and the α -carbon atom of the amino acid involved, namely α -hydroxyalanine. This results in a pyruvoyl precursor of phenylalanine-proline lactam which can have structure 2 or 3. Structure 3 is that of pyroergotamine, a reference sample of which showed the same GC and MS behaviour as the above pyruvoyl precursor. However, this does not preclude structure 2 (which is more stable) for this precursor, but for which no reference sample was available. Structure 4 is the phenylalanine-proline lactam, which is obtained in two forms, namely L-phe-D-pro lactam and L-phe-L-pro lactam.

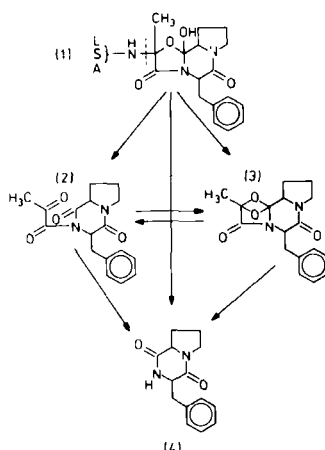
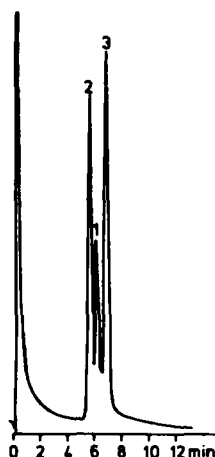


FIGURE 18.6

GAS CHROMATOGRAM OF ERGOTAMINE BASE¹³

on a 1.8 m by 2 mm glass column, packed with 3 % SE-30 on Chromosorb W HP at 225°C.

1 = L-phe-L-pro lactam, 2 = L-phe-D-pro lactam, 3 = pyruvoyl precursor of L-phe-D-pro lactam



The other Ergot-peptide alkaloids: ergosine, ergostine, ergocristine, ergokryptine and ergocornine, showed similar decomposition patterns in the injection port, in that they all gave cyclic lactams containing two amino acids and a precursor of these lactams, which, besides these two amino acids, contained a deaminated third hydroxyamino acid. This was confirmed by GC-MS. Table 18.4 summarizes the various degradation products, their retention times and retention indices on SE-30 and their quasi-molecular ions in CI-MS. Figure 18.7 shows a gas chromatogram of a mixture of the six Ergot-peptide alkaloids, injected as free bases in ethanol: each individual alkaloid can be identified by the presence of two or three characteristic degradation products.

Plomp et al.¹⁴ investigated further the thermal decomposition of the dihydroergotoxine alkaloids for quantitative purposes and they found that the reaction is not catalyzed by a metal surface as stated by Szepesi and Gazdag¹¹. They used an all-glass system and a selective and sensitive nitrogen detector and obtained a sensitivity of about 1-10 ng for the various dihydroergotoxine alkaloids. Using a combination of GC and chemical ionization (CI) mass fragmentography, a 10-fold increase in sensitivity, compared with the GC method used, was obtained. By means of electron impact (EI) and chemical ionization mass spectrometry, the mechanism of the thermal decomposition of the dihydroergotoxine alkaloids was elucidated.

The relative retention times of the decomposition products of the dihydroergotoxine alkaloids are given in Table 18.5. The influence of the injection port temperature on the decomposition of the alkaloids is illustrated in Figure 18.9. The reproducibility of the method is good, as can be seen from Table 18.6.

TABLE 18.4

GLC DEGRADATION PRODUCTS, RETENTION TIMES, RETENTION INDICES AND QUASI-MOLECULAR IONS OF SOME ERGOT PEPTIDE ALKALOIDS¹³

Alkaloid	Amino acids in peptide moiety		Degradation products detectable by GLC	Peak *)	t _R (min)	Ret. index	MH ⁺ (CI-MS)
Ergotamine	α-hydroxy-Ala	Phe Pro	L-Phe-L-Pro lactam	1	6.33	2300	245
			L-Phe-D-Pro lactam	2	5.76	2275	245
			pyruvoyl-Phe-Pro lactam **)	3	7.00	2340	315
Ergosine	α-hydroxy-Ala	Leu Pro	Leu-Pro lactam	4	1.93	1900	211
			pyruvoyl-Leu-Pro lactam **)	5	2.81	2075	281
Ergostine	α-hydroxy-α-aminobutyric acid	Phe Pro	L-Phe-L-Pro lactam	1	6.33	2300	245
			L-Phe-D-Pro lactam	2	5.76	2275	245
Ergocristine	α-hydroxy-Val	Phe Pro	α-ketobutyryl-Phe-Pro lactam **)	6	9.20	2435	329
			L-Phe-L-Pro lactam	1	6.33	2300	245
			L-Phe-D-Pro lactam	2	5.76	2275	245
Ergokryptine	α-hydroxy-Val	Leu Pro	α-ketoisovaleryl-Phe-Pro lactam **)	7	10.58	2480	343
			Leu-Pro lactam	4	1.93	1900	211
Ergocornine	α-hydroxy-Val	Val Pro	α-ketoisovaleryl-Leu-Pro lactam	8	4.28	2175	309
			Val-Pro lactam	9	1.51	1810	197
			α-ketoisovaleryl-Val-Pro lactam	10	3.55	2100	295

*) Peak number in Figure 18.7, **) The exact structure of this component is unknown at the present time. As indicated in Figure 18.5, pyruvoyl-Phe-Pro lactam may be present as an α,β-diketo structure (2) or as a structure with a dioxane ring (3). This also applies to the other deaminated tripeptide lactam components in this table.

FIGURE 18.7

GAS CHROMATOGRAM OF SIX ERGOT-PEPTIDE ALKALOIDS¹³

on SE-30 (see Figure 18.6). For peak numbers, see Table 18.4

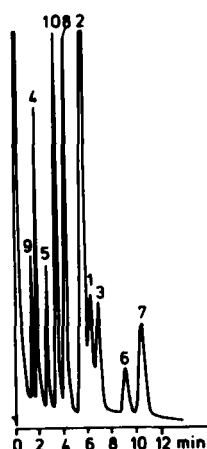


FIGURE 18.8

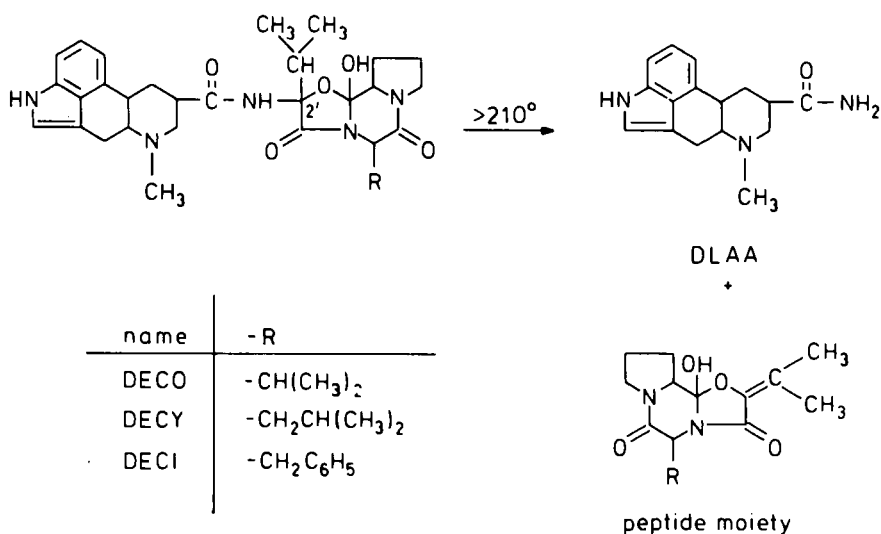
STRUCTURES OF DIHYDROERGOTOXINE ALKALOIDS AND THEIR THERMAL DECOMPOSITION PRODUCTS¹⁴

TABLE 18.5

RELATIVE RETENTION TIMES OF DECOMPOSITION PRODUCTS OF DIHYDROERGOTOXINE ALKALOIDS¹⁴

on a glass column, 1.25 m x 3.8 mm I.D., packed with 3 % SE-30 on Supelcoport, at 200-270°C, temperature programming

Compound	Relative retention time
Internal standard	1.00 ($t_R = 12.67$ min)
Dihydroergocornine	0.46
Dihydroergokryptine	0.57
Dihydroergocristine	1.42
Dihydrolysergic acid amide	2.42

Application of the method to the determination of the dihydroergotoxine alkaloids in commercial samples and pharmaceutical preparations were all carried out with the free base of the alkaloids since analysis of their salts cannot be performed without excessive decomposition. The mean dihydroergotoxine methane sulphonate content of five commercial samples was 95.2 ± 2.9 %, including a mean dihydroergocornine content of 30.9 ± 0.8 %, a mean dihydroergokryptine content of 32.8 ± 2.1 % and a mean dihydroergocristine content of 31.5 ± 3.5 %. Almost the same data were observed for dihydroergotoxine tablets obtained from two manufacturers.

FIGURE 18.9

GAS CHROMATOGRAM OF DIHYDROERGOTOXINE ALKALOIDS¹⁴

GLC conditions, see Table 18.5. 1 = Dihydroergocornine, 2 = dihydroergokryptine, 3 = dihydroergocristine, 4 = internal standard.

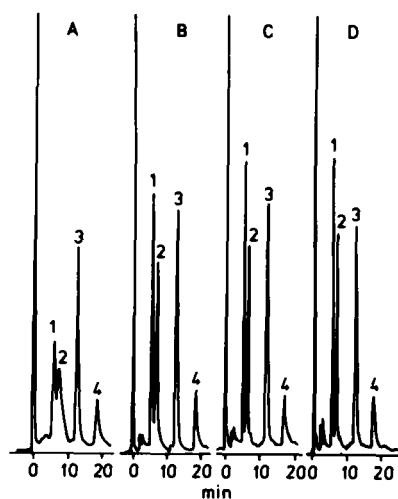


TABLE 18.6

RESULTS OF REPRODUCIBILITY STUDIES¹⁴

Concentration of compound ($\mu\text{g}/\mu\text{l}$)	Standard deviation (%)		
	Dihydroergocornine	Dihydroergokryptine	Dihydroergocristine
0.10	6	11	16
0.20	3	6	6
0.50	2	2	4
1.00	1	1	3
3.00	1	2	3

TABLE 18.7

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF ERGOT ALKALOIDS

Column	Solid support mesh	Stat.phase	%	Temperature	Comp. Prep.	Ref.
s.s., 1.5 m x 3 mm I.D.	MGB	SE-30	0.2	270°C	LSD hy.id.	1
glass, 1.8 m x 3 mm	MGB	SE-30	0.3	280°C	LSD id.	2
glass		SE-52	2	245°C	LSD id.	3
					LSD TMS id.	
glass, 3 ft	CW 80-100	SE-30	3	225°C	ly.am.	4
				250°C	ly.am.TMS	

TABLE 18.7 (continued)

Column	Solid support mesh	Stat.phase	%	Temperature	Comp.Prepare.	Ref.
		SE-52	2	250°C	hal.LSD.id.	5
		-	2	245°C	hal.LSD.TMS.id.	5
glass, 1.8 m x 3 mm I.D.	CW AWS 80-100	OV-17	1.5	270°C	LSD.ocd.id.	6
glass, 6 ft x 2 mm I.D.	TMGB 100-120	OV-17	0.25	258°C	LSD.isom.TMS.	7
glass, 6 ft x 3 mm	GQ AWS 100-120	JxR	3	240°C	clav.alk.der.	8
-	GP AWS 100-120	SE-30	5	265°C	s.	8
-	GP AWS 100-120	XE-60	5	225°C		
glass, 1.2 m x 6.4 mm O.D.	GQ 80-100	OV-1	1	260°C	ergm.TMS.qnt.	9
glass, 5 ft x 3 mm	CW AWS 80-100	SE-30	10	210°C		
-	-	SE-52	3	198°C pr	agr.TFA.qnt.	10
-	-	OV-17	10	220°C	agr.TMS.qnt.	10
s.s., 1 m x 3.2 mm	GQ 80-100	Dex.	2	180-280°C pr	dh.ergt.	11
				200-300°C pr	decp.	11
glass, 1.8 m x 2 mm I.D.	CG HP 80-100	SE-30	3	225°C	ertm.decp.id.	12
-	-	SP 2250	3	225°C	tox.	
glass S, 1.8 m x 2 mm I.D.	CG HP 80-100	SE-30	3	225°C	erg.pept.alk.	13
					decp.id.	
glass, 1.25 m x 3.8 mm I.D.	Sup. 80-100	SE-30	3	200-270°C pr	ergt.alk.qnt.	14
					decp.	

TABLE 18.8

ERGOT ALKALOIDS - LIST OF ABBREVIATIONS

agr = agroclavine
 alk = alkaloid
 AW = acid washed
 clav = clavine
 CG = Chromosorb G
 CW = Chromosorb W
 decp = decomposition product
 der = derivative
 Dex = Dexsil 300
 dh.ergt = dihydroergotamine
 erg.pept = ergot peptide
 ergt = ergotamine
 ergm = ergometrine
 ertm = ergotamine
 GP = Gas Chrom P
 GQ = Gas Chrom Q
 hal = hallucinogenic drugs
 HP = high performance
 hy = hydrogenated

I.D. = inside diameter
 id = identification
 isom = isomer
 LSD = lysergic acid diethylamide
 ly.am = lysergic acid amide
 MGB = micro glass beads
 ocd = on-column derivatization
 O.D. = outside diameter
 pr = (temperature) programming
 s = separation
 S = silanized
 s.s = stainless steel
 Sup = Supelcoport
 TFA = trifluoroacetyl
 TMGB = textured micro glass beads
 TMS = trimethylsilyl
 tox = toxicology

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II.6 STEROIDAL ALKALOIDS (GLYCOALKALOIDS)

Chapter 19

SOLANUM ALKALOIDS

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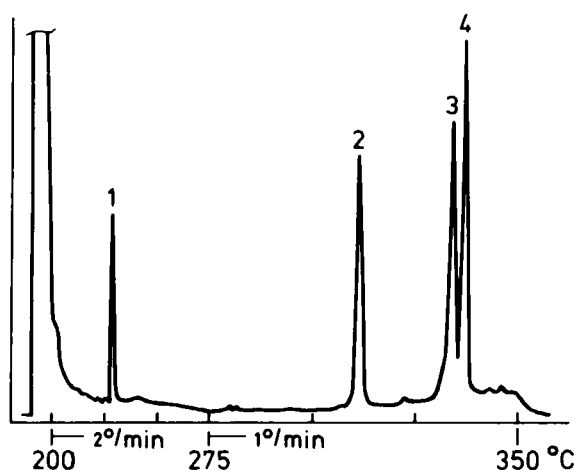
19.1. *SOLANUM* ALKALOIDS

To be able to gas chromatograph the high molecular weight glycoalkaloids present in potatoes (solanine, m.w. 869, demissine, m.w. 1034) Herb et al.¹ permethylated the glycoalkaloids to improve their volatility for gas chromatographic analysis. Permethylated derivatives of glycoalkaloids were preferred to trimethylsilyl derivatives because of the greater volatility and lower molecular weights of the former. The alkaloids were extracted from samples of 20 g ground fresh potatoes with chloroform-methanol (1:2). After addition of an aqueous solution of 0.8 % sodium sulphate, the alkaloids were found in the methanol layer. This was separated from the rest and evaporated to dryness. The glycoalkaloids were extracted with absolute methanol and the permethylation performed with methyl sulphate, sodium hydride and methyl iodide. The permethylated compounds were extracted with benzene and the gas chromatography performed on a packed 3 % Dexsil 300 column and an 3 % OV-1 column. With the OV-1 column it was found that the glycoalkaloids could be eluted at temperatures 25-30° lower than on the Dexsil 300 column, with equivalent separation.

FIGURE 19.1

CHROMATOGRAM OF PERMETHYLATED GLYCOALKALOIDS¹

on a 90 cm by 2 mm glass column, packed with 3 % Dexsil 300 on Supelcoport; temperature programming 275-350°C. 1 = solanidine, 2 = β -chaconine, 3 = α -chaconine, 4 = α -solanine.



Because of the relative high column temperature that had to be used (Dexsil 300: 275-350°C by temperature programming; OV-1: 330°C isothermal) bleeding took place and also deterioration of the column. This resulted in poor separation and the formation of artefacts after some days or weeks of use of the column. A chromatogram of some permethylated glycoalkaloids is found in Figure 19.1 and the relative retention and the retention temperatures in Table 19.1.

TABLE 19.1

RELATIVE RETENTION AND RETENTION TEMPERATURES OF PERMETHYLATED GLYCOALKALOIDS¹

on a 3 % OV-1 packed column on Gas Chrom Q, 120 cm by 2 mm, at 330°C. Retention relative to α -solanine, time of elution 12 min.

Alkaloid (permethylated)	Relative retention	Retention temperature, °C
Solanidine		223
β -Chaconine	0.35	303
α -Chaconine	0.87	324
α -Solanine	1.00	327
Demissine	2.84	330
Tomatine	4.23	330

TABLE 19.2

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF STEROIDAL ALKALOIDS (GLYCOALKALOIDS)

Column	Solid support mesh	Stat.phase	%	Temperature	Comp.Preparation	Ref.
glass, 90 cm x 2 mm	Sup. 100-120	Dex.	3	275-350°C pr	prm.alk.s.	1
glass, 120 cm x 2 mm	GQ 100-120	OV-1	3	330°C		

Abbreviations: Sup = Supelcoport, GQ = Gas Chrom Q, Dex = Dexsil 300, pr = (temperature) programming, prm = permethylated, alk = alkaloid, s = separation

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II.7 XANTHINE ALKALOIDS

Chapter 20

XANTHINE ALKALOIDS

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20.1. XANTHINE ALKALOIDS

Several authors have included one or more naturally occurring xanthine derivatives (caffeine, theobromine and theophylline) in their gas chromatographic separation of alkaloids. Lloyd et al.¹ used a packed column with SE-30 on Chromosorb W, so did Brochmann-Hanssen and Baerheim Svendsen², Parker et al.³ and Kazyak and Knoblock⁴. Kolb and Patt⁵ introduced more polar stationary phases (neopentyl glycol sebacate). Brochmann-Hanssen and Fontan^{6,7} applied various polar stationary phases (EGSS-Y and HI-EFF-8B) and such phases on polyvinylpyrrolidone treated support. Jain and Kirk⁸ used HI-EFF-8B for the separation of caffeine and theobromine.

The first systematic investigation on the gas chromatographic separation of xanthine derivatives was published by Reisch and Walker⁹. The naturally occurring xanthines, caffeine, theobromine and theophylline, as well as a number of derivatives, were gas chromatographed on an 1.5 % packed SE-30 column on Chromosorb W. In Table 20.1 the compounds are given and in Figure 20.1 typical gas chromatograms.

Kamei and Atsushi¹⁰ chromatographed xanthine derivatives on packed columns with SE-30, SE-52, QF-1, OV-1, OV-17 and XE-60 as stationary phases. Caffeine, theobromine, theophylline, oxyethyltheophylline, oxypropyltheophylline and dyphylline were investigated. Mass spectrometry was carried out with an on-line Hitachi 002-type high resolution mass system. The relative retention times of the compounds investigated are given in Table 20.2. The TMS- and TFA-derivatives of the methylated xanthines had good gas chromatographic properties. Typical chromatograms are given in Figure 20.2.

In their publication on the gas chromatography of alkaloids on capillary columns, Massingill and Hodgkins¹¹ also chromatographed caffeine. A 100 feet long capillary coated with

TABLE 20.1

XANTHINE DERIVATIVES GAS CHROMATOGRAPHED BY REISCH AND WALKER⁹

- 1 = Caffeine
- 2 = Theobromine
- 3 = Theophylline
- 4 = 7-(2'-Hydroxyethyl)-theophylline
- 5 = 1-(2'-Hydroxypropyl)-theobromine
- 6 = 7-(N-methyl-N-hydroxyethyl)-3-amino-2-hydroxypropyl)-theophylline
- 7 = 7-(2,3-Dihydroxypropyl)-theophylline
- 8 = 1-Allyltheobromine
- 9 = 7-Propin-(2')-yltheophylline
- 10 = 1-Propin-(2')-yltheobromine
- 11 = 1-n-Hexyltheobromine
- 12 = 8-Chlorotheophylline

FIGURE 20.1

GAS CHROMATOGRAMS OF XANTHINE DERIVATIVES LISTED IN TABLE 20.1⁹

on a 6 ft by 1/4 inch packed column, with 1.5 % SE-30 on Chromosorb W. (1) in diethylamin solution and temperature programming 200-300°C, (2) in aqueous solution and temperature programming 200-300°C and (3) in chloroform solution, 7 min isothermal, then temperature programming to 250°C. For peak numbers, see Table 20.1.

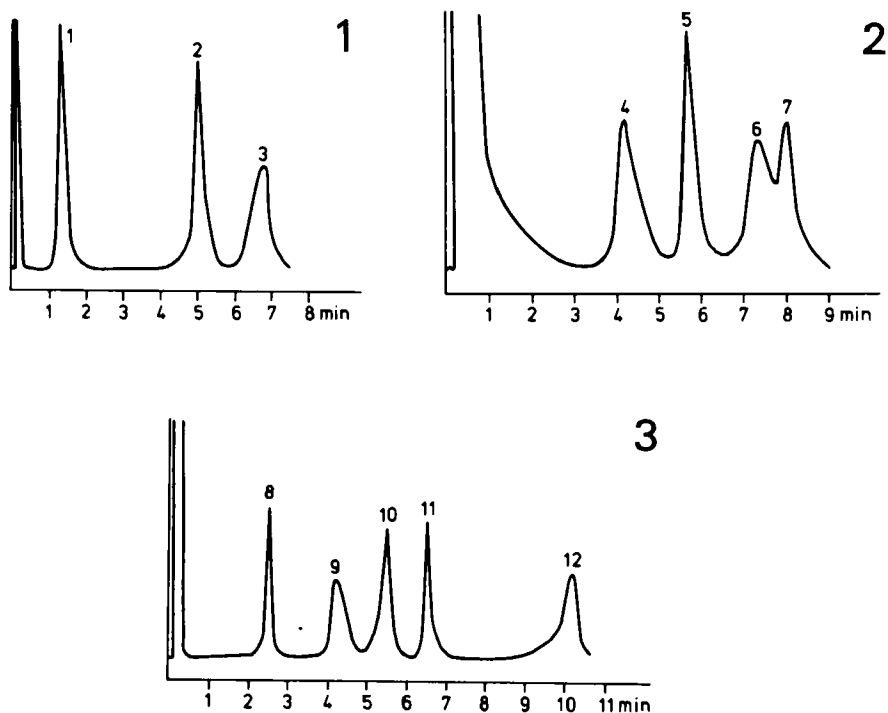


TABLE 20.2

RELATIVE RETENTION TIMES OF METHYLATED XANTHINES¹⁰

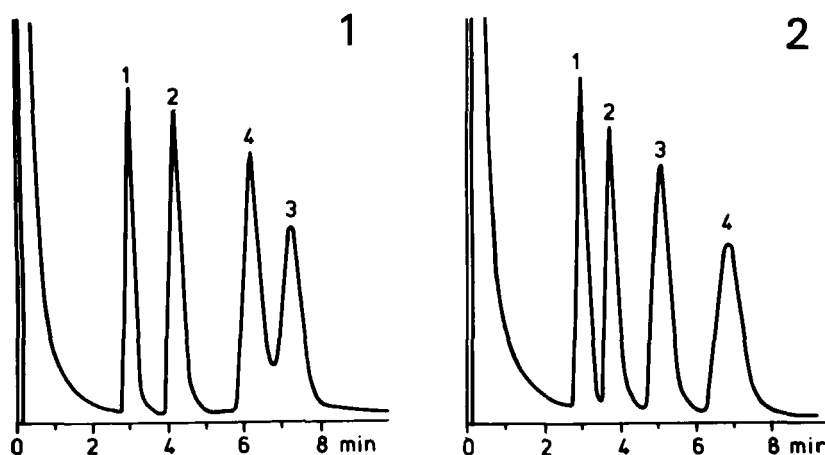
on stainless steel columns, 1 m by 3 mm, packed with various stationary phases on Chromosorb W. at different temperatures

Compound	1.5 % SE-30 190°C	3 % SE-30 200°C	1.5 % SE-52 210°C	3 % QF-1 210°C	2 % OV-1 205°C	2 % OV-17 240°C	3 % XE-30 230°C
Caffeine	0.43	0.42	0.45	0.46	0.49	0.45	0.32
Theobromine	0.61	0.82	0.61	0.77	0.77	0.54	0.57
Theophylline	1.02	1.00	0.95	0.85	0.80	0.81	1.19
Oxypropyltheophylline	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Oxyethyltheophylline	1.15	1.03	1.16	1.18	1.29	1.19	1.38
Dyphylline	3.29	3.82	2.75	2.51	3.23	2.49	3.78

FIGURE 20.2

GAS CHROMATOGRAMS OF (1) TFA AND (2) TMS DERIVATIVES OF METHYLATED XANTHINES¹⁰

on a stainless steel column, treated with HMDS, 1 m by 3 mm, packed with 3 % XE-60 on Chromosorb W, 220°C. 1 = oxypropyltheophylline, 2 = oxyethyltheophylline, 3 = dyphylline, 4 = internal standard (dioctylphthalate)



QF-1, a 200 feet long one coated with SE-30 and a 100 feet long one coated with Apiezon L were used with temperature programming up to 250°C. On the Apiezon L column caffeine was not eluted. Bohn et al.¹² used a glass capillary coated with Triton X 305 for a study on illicit heroin samples, which regularly contained caffeine. Caffeine was eluted as a sharp peak.

Christophersen and Rasmussen¹³ included caffeine in a study on glass capillary gas chromatography of narcotic drugs, using SE-30 as stationary phase and temperature programming up to 250°C.

Polysiloxane deactivated capillaries - glass and fused silica - coated with Carbowax 20 M

or CP-Sil 5 (a dimethylpolysiloxane phase prepared from SE-30) were used by Schepers et al.¹⁴ for the analysis of drugs including alkaloids such as caffeine and theophylline.

Because of the extensive use of theophylline for the treatment of bronchial asthma and other cardiorespiratory disorders, many investigations have been carried out by means of gas chromatography to determine theophylline in serum, plasma and saliva. In many cases theophylline has been gas chromatographed as such, in some other cases after derivatization. Methyl derivatives^{15,16,17,18}, *n*-butyl derivatives^{19,20,21,22,23,24,25,26}, propyl derivatives²⁷, pentyl derivatives^{28,29} and pentafluorobenzyl derivatives^{30,31} have been made in order to obtain better gas chromatographic properties of theophylline and thus better detection possibilities. A number of stationary phases have been applied, from non-polar ones (SE-30) to very polar ones (HI-EFF 8B). OV-17 is the stationary phase that has mostly been used.

20.2. CAFFEINE

20.2.1. Caffeine in coffee, tea and beverages

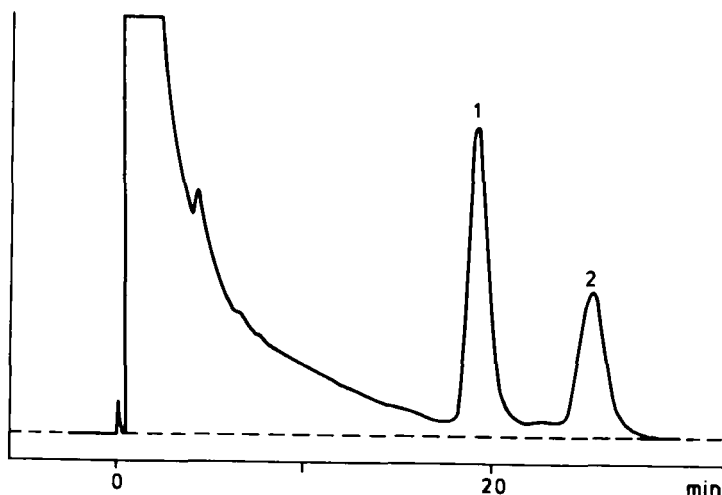
A great number of methods have been developed for the determination of caffeine in coffee, e.g. gravimetric, spectrophotometric, iodometric and colourimetric methods. Vitzthum³² applied gas chromatography for such determinations. He developed a method for the determination of caffeine in decaffeinated coffee.

Because gas chromatography of caffeine on packed columns of 2.5 % SE-30 on Chromosorb gave tailing, Vitzthum³² preferred to use a packed column of 0.2 % SE-30 on micro-glass beads. After extraction of the coffee-extract with 96 % ethanol under reflux, the ethanolic extract was gas chromatographed directly, using pyrene as an internal standard. A typical chromatogram is shown in Figure 20.3.

FIGURE 20.3

CHROMATOGRAM OF CAFFEINE IN DECAFFEINATED SOLUBLE COFFEE³²

on a 2 m long packed column of 0.2 % SE-30 on micro-glass beads at 210°C. 1 = caffeine, 2 = pyrene (internal standard).



Good agreement was found between the results obtained by means of gas chromatography and by UV-spectrophotometry. However, the gas chromatographic method is more suitable for rapid determinations.

Newton³³ developed a gas chromatographic method for the determination of caffeine in instant tea, using the extraction and clean-up procedure of Yeransian et al.³⁴, prior to the gas chromatographic analysis. This was carried out on a packed column of 10 % DC-200 on Gas Chrom Q at 190°C using a thermoionic KCl detector. This detector is sensitive to as little as 1 ng caffeine. Comparative studies showed that the results obtained with the gas chromatographic method and with the UV-spectroscopic method of Yeransian et al.³⁴ were in good agreement.

Fogden and Urry³⁵ determined caffeine in cola drinks after a thorough extraction of the caffeine from alkalized cola (ammonia) with dichloromethane, concentration of the solution, and gas chromatography on a packed column of Versamide 930 2 % on Phasesep N using procaine as an internal standard. The method gives a recovery of 95 to 98 % of the caffeine added to cola preparations.

Schilling and Gal³⁶ described methods for all types of marketed coffee products. Coffee was extracted with chloroform and the chloroform solution was gas chromatographed directly using pyrene as an internal standard. A packed column of 10 % SE-30 on Varaport was used for the analysis. Sharp peaks were obtained, very suitable for quantitative determinations.

A rapid method to assay caffeine in commercial coffee samples was developed by Vitzthum et al.³⁷. Aqueous extracts were automatically gas chromatographed directly, using 5-aminoquinoline as an internal standard. A packed column of Carbowax 20 M on Chromosorb G HP was used. The analysis was carried out at 220°C using a nitrogen sensitive flame ionization detector. For coffee, a sample of 150 mg was ground, mixed with magnesium oxide and boiled for 10 min. with 50 ml of water. After filtration the filter was washed with 10 ml hot water three times. A solution of the internal standard was added and the total volume brought to 100.0 ml. 2 µl of the solution was used for the gas chromatographic analysis. The main advantage of the method is the extraction with water, which is rapid and complete.

Bandion³⁸ described a method for the assay of caffeine and quinine in beverages. The alkaloids were extracted from the alkalized drink (NaOH) with chloroform containing the internal standard (pyrene); the solvent was evaporated and the residue dissolved in pyrene-free chloroform. An aliquot was used for the gas chromatographic analysis. Alcoholic beverages were diluted with water before extraction with chloroform (containing pyrene). In the range of 50-200 mg caffeine per litre of non-alcoholic beverages, an accuracy of ± 3.6 % was obtained and for alcoholic beverages (coffee liqueur) it was between - 2.4 and + 3.6 %. Detection limit was 4 mg caffeine per litre.

20.2.2. Caffeine in plasma

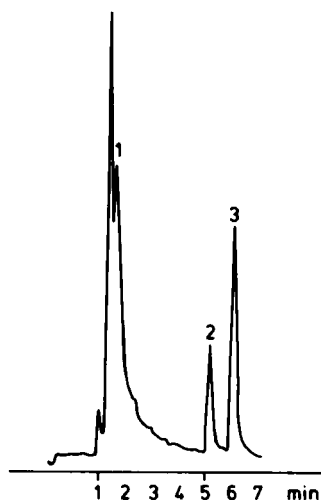
To overcome the major drawbacks of previous methods for the determination of caffeine in body fluids (difficult isolation from interfering materials, a substantial blank error and low sensitivity) Grab and Reinstein³⁹ developed a gas chromatographic method for such determinations. The method involved extraction of caffeine from plasma samples (2 ml) with chloroform after the aqueous phase was adjusted to pH 11.5-12.0. The chloroform extract was evaporated to dryness and redissolved in carbon disulphide. Hexobarbital was used as an internal

standard, and the gas chromatographic analysis carried out on a packed column of 3 % OV-17 on silanized Chromosorb W AW at 200°C. Caffeine was determined at a concentration of 0.25 µg per ml. Recovery of caffeine added to plasma samples (0.5-1.5 µg/ml) was 98-102 %. A typical chromatogram is shown in Figure 20.4.

FIGURE 20.4

CHROMATOGRAM OF CAFFEINE IN PLASMA³⁹

on a 3 % OV-17 column, 6 feet by 1/8 inch O.D., at 200°C; 1 = carbon disulphide, 2 = hexobarbital, 3 = caffeine.



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Merriman et al.⁴⁰ improved the method of Grab and Reinstein³⁹. They developed a gas chromatographic-mass spectrometric micromethod. As little as 20 ng of caffeine can be measured, and, therefore accurate estimates of caffeine concentrations in 100 µl or less of biological samples can be made. The method is rapid, specific and sensitive. A packed column of 3 % Dexsil 300 on Chromosorb Q was used at 210°C, and glutethimide was used as an internal standard.

Blood samples (0.1 ml) were mixed with 0.2 ml of 0.9 % Na Cl solution and 0.5 ml of glutethimide in chloroform solution. The mixture was mixed for 60 seconds and the phases separated by low-speed centrifuging. All the caffeine and glutethimide partitioned into the chloroform phase. The chloroform phase was evaporated to dryness, dissolved in 0.5 ml of acetone and gas chromatographed. The caffeine concentrations relative to the glutethimide concentrations were determined by monitoring the molecular ion of caffeine (m/e 194) to the M-28 ion of glutethimide (m/e 189).

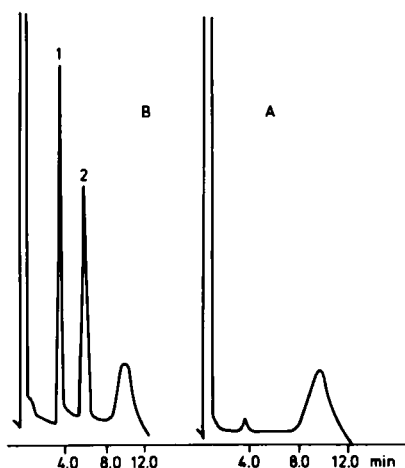
The increased sensitivity and selectivity of the alkali flame detector for nitrogen-containing compounds, led to the development of an assay for caffeine in plasma, by Cohen et al.⁴¹. Amounts down to 0.25 µg/ml plasma can be determined.

Plasma samples of 1.0 ml were alkalinized (NaOH), the internal standard (mepivacaine) added and the mixture extracted with chloroform. The chloroform solution was evaporated and the residue redissolved in methanol (25 μ l). 1.5 μ l of the methanol solution was injected for the gas chromatographic analysis on a packed column of 3 % OV-17 on Chromosorb P. A typical chromatogram is given in Figure 20.5. Peak height ratio measurements produced linear standard curves in the 0.25-10.0 μ g/ml range. Absolute sensitivity from a 1.0 ml plasma sample was 0.1 μ g/ml. The relative deviation of a 2.0 μ g/ml pooled plasma standard curve (done repeatedly over several months) was 5.2 %.

FIGURE 20.5

CHROMATOGRAM OF HUMAN PLASMA SAMPLES⁴¹

on a 3 % OV-17 packed column on Chromosorb P, 1.82 m by 2.5 mm I.D., at 210°C. A = pooled plasma blank, B = plasma from a normal volunteer containing 5 μ g of caffeine (1) and 12 μ g of mepivacaine/ml (2).



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In a comparative study of micromethods for the determination of caffeine in small plasma samples (10-50 μ l) a gas chromatographic and a radioactive labelling method were used by Milon and Antonioli⁴². The caffeine was extracted into chloroform and the gas chromatography carried out on a packed column using 3 % SE-30 on Chromosorb W, and (1-CH₃¹⁴C) caffeine as an internal standard. Because the caffeine peak had an asymmetrical form, formic acid vapour was added to the carrier gas, as proposed by Welton⁴³. The reproducibility of the gas chromatographic method for the range 1-20 mg/litre of caffeine, added to plasma, was found to be better than 5 %.

Bradbrook et al.⁴⁴ described a method for the assay of caffeine in 0.5 ml samples of plasma. The sample was made alkaline with NaOH and after addition of the internal standard (phenacetin) the extraction was achieved with 3 ml of ethyl acetate by means of vortex mixing followed by centrifuging. The residue of the ethyl acetate solution was dissolved in 20 μ l

methanol and 1-3 μ l used for the gas chromatographic analysis on packed columns of Apiezon M 10 % or Poly S-179 3 % at 225°C. Comparison of a thin layer chromatographic method with the gas chromatographic method showed that the gas chromatographic method required more complex sample preparation, but had greater sensitivity than the thin layer chromatographic method. However, both methods were suitable for use in studies of caffeine in pharmacokinetics.

20.2.3. Caffeine in pharmaceutical preparations

Caffeine is often present in pharmaceutical preparations in combination with other drugs, such as acetylsalicylic acid, phenacetin, antipyrin, etc. Because classical analytical techniques (e.g. spectrophotometric and colourimetric methods) can be quite time consuming and leave much to be desired in accuracy and precision, gas chromatography has been quite extensively applied for the analysis of such multicomponent preparations.

In 1963 Hoffman and Mitchell⁴⁵ described a method for determining in a single run the active ingredients in APC-tablets (= Acetylsalicylic acid, Phenacetin, Caffeine tablets). Due to the high ratio of phenacetin to caffeine (162 mg : 32 mg) and the small differences in their retention times, it was not very satisfactory for the determination of caffeine. However, the means of several analyses yielded results close to either the labeled claim or the composition by synthesis. The results of a series of analyses of synthetic APC mixtures are listed in Table 20.3.

TABLE 20.3

PRECISION AND ACCURACY ANALYSIS OF A SYNTHETIC APC MIXTURE⁴⁵

on a packed column of DC 200, 2 % on Haloport F, 6 feet by 4 mm I.D. and temperature programming from 75°C to 200°C

Component	Composition by syntheses. % by wt.	Composition by syntheses						Av. Deviation from Mean %	Av. Error %
		Run 1	Run 2	Run 3	Run 4	Mean			
Synthetic mixture	Acetylsalic. acid	53.52	53.31	55.06	54.33	54.75	54.36 \pm 0.6	+ 1.6	
	Acetophenet- idin	38.34	38.36	37.12	37.74	37.96	37.79 - 1.0	- 1.4	
	Caffeine	7.65	8.31	7.81	7.92	7.28	7.83 \pm 3.6	+ 2.3	
Tablet Assays									
Tablet A	Acetylsalic. acid	Labeled composition mg	Run 1 mg	Run 2 mg	Run 3 mg	Run 4 mg	Run 5 mg	Run 6 mg	Mean mg
	Acetophenet- idin	226.8	223.53	216.14	221.56	235.10	221.17	222.10	223.14
	Caffeine	162.0	168.35	172.61	167.92	162.66	166.29	166.92	167.45
Tablet B	Acetylsalic. acid	32.4	32.4	32.4	31.72	31.34	33.70	32.10	32.28
	Acetophenet- idin	226.8	224.64	217.04	226.36	227.70	225.23		224.19
	Caffeine	162.0	161.78	170.21	161.48	160.00	162.16		163.11
		32.4	34.75	33.87	33.28	33.45	33.78		33.85

Two microliter injections of chloroformic solutions were used throughout.

Haefelfinger et al.⁴⁶ developed a similar method for the determination of phenacetin, isopropylantipyrin, caffeine and persedon. A powdered tablet was brought into a 200 ml columetric flask, Sedormid (= allyl-isopropylacetyl urea) was added as an internal standard, and acetone to 200 ml. 1 μ l of the solution was gas chromatographed. A comparative study of a thin-layer chromatographic method and an ion exchange resin separation, in combination with UV-determination, and the gas chromatographic method, showed that the latter was the more elegant and rapid. A packed column of 10 % SE-30 on Celite was used for the gas chromatography, which operated at 215°C to assay caffeine in the multicomponent preparations mentioned.

Dechene et al.⁴⁷ determined acetylsalicylic acid, phenacetin, caffeine and codeine in tablets. Acetylsalicylic acid and phenacetin were separated and determined on one packed column of DS 200 on Haloport by temperature programming, 100-180°C, whereas caffeine and codeine were separated on a packed column of SE-30 (10 %) on Chromosorb W AW by temperature programming, 195-260°C. Codeine and caffeine were extracted from the powdered tablet material with chloroform after the addition of alkali, and the chloroform solution used for the gas chromatographic assay. Results obtained with the method are listed in Table 20.4.

TABLE 20.4

RESULTS OF GAS CHROMATOGRAPHIC ANALYSES OF APC TABLETS⁴⁷

Ingredient assay	Recovery (%)			
	Acetylsalic. acid	Phenacetin	Caffeine	Codeine phosphate
Average of 10 assays	98.30	100.77	100.0	102.88
High value	103.30	106.20	105.04	109.60
Low value	93.76	95.46	97.92	94.06
Standard deviation	± 2.77	± 3.26	± 1.16	± 4.83

The sensitive thermoionic KCl detector used by Newton³³ for the determination of very low concentrations of caffeine in instant tea, was also used by Alber and Overton⁴⁸ for the determination of caffeine in tablets containing salicylamide and acetaminophen. The tablet powder was extracted with acetone after addition of the internal standards (amobarbital and cyclizine) and the solution used for the gas chromatographic analysis. This was carried out on a packed column of OV-17 3 % on Gas Chrom Q at 165°C. The signals from the KCl thermoionic detector were amplified and fed directly into the analog-to-digital converter of a PDP 12 A LINC system computer. The results obtained by means of the computer were compared to manual peak height measurements for 6 commercial preparations injected in duplicate, and to results obtained from a UV-procedure. A typical chromatogram is given in Figure 20.6 and the results obtained are listed in Table 20.5.

The gas chromatographic method is particularly well suited for multiple analyses, which can be applied to automated techniques and individual tablet analysis.

Because drug assay in suppositories following the classical techniques (e.g. spectrophotometric and colourimetric methods) is cumbersome and not very accurate or precise due to interference of the excipients and other additives, Cometti et al.⁴⁹ applied gas chromatography for such assays. The suppositories were dissolved in ethanol or chloroform, containing the internal standards to be used and depending on the drugs present. The gas chromatography was carried out on different packed columns, depending on the drugs to be determined. Typical

gas chromatograms of suppositories containing caffeine and some other drugs are given in Figure 20.7. Analytical results of some suppositories are listed in Table 20.6.

FIGURE 20.6

GAS CHROMATOGRAM OF SALICYLAMIDE (1), AMOBARBITAL (2), ACETAMINOPHEN (3), CAFFEINE (4) AND CYCLIZINE (5)⁴⁸
on a 3 % OV-17 packed column on Gas Chrom Q at 165°C

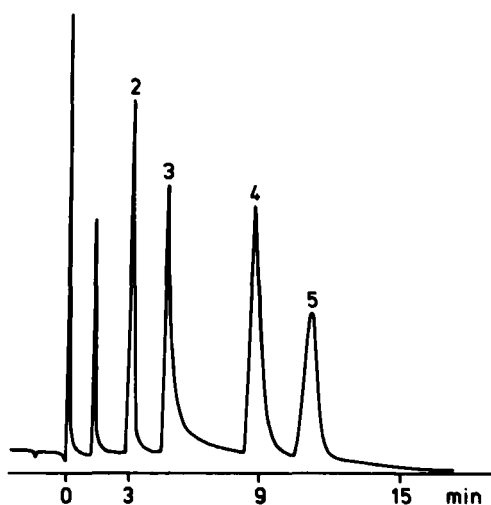


TABLE 20.5

COMPARISON OF GLC, GLC-COMPUTER, AND UV ASSAY RESULTS OF SOME FORMULATIONS⁴⁸

Compound	Declared mg/tablet	Manual peak ht.		Computer peak ht.		UV spectrophotometry	
		mg/tablet	% of de- clared	mg/tablet	% of de- clared	mg/tablet	% of de- clared
Salicylamide	250	249	99.6	251	100	249.7	99.88
Acetaminophen	250	241	96.4	243	97.2	249.4	99.76
Caffeine	18.0	18.8	104	18.6	103	-	-
Salicylamide	200	204	102	200	100	204.3	102.2
Acetaminophen	120	124	103	124	103	120.0	100.0
Caffeine	30	32.5	108	32.4	108	-	-
Salicylamide	180	175	97.2	176	97.8	189	105
Acetaminophen	180	182	101	180	100	185	103
Caffeine	30	31.9	106	31.6	105	30.9	103
Salicylamide	230	227	98.7	224	97.4	223.2	97.1
Acetaminophen	150	158	105	157	105	150.7	100.5
Caffeine	30	28.4	94.7	27.3	91.0	25.1	83.6

FIGURE 20.7

CHROMATOGRAMS OF SUPPOSITORIES⁴⁹

Left: 1 = diphenylamine (internal standard), 2 = lidocaine, 3 = chlorpheniramine, 4 = d-propoxyphene, 5 = caffeine on a packed column of 2 % Carbowax 20 M and 2 % SE-30 on Gas Chrom P, impregnated with 5 % KOH, at 195°C. Right: 1 = phenacetin, 2 = caffeine, 3 = aminophenazone, 4 = chlorpheniramine and 5 = chlorothioxantone (internal standard) on a packed column of 10 % UCC W98 on Diatoport S, at 205°C

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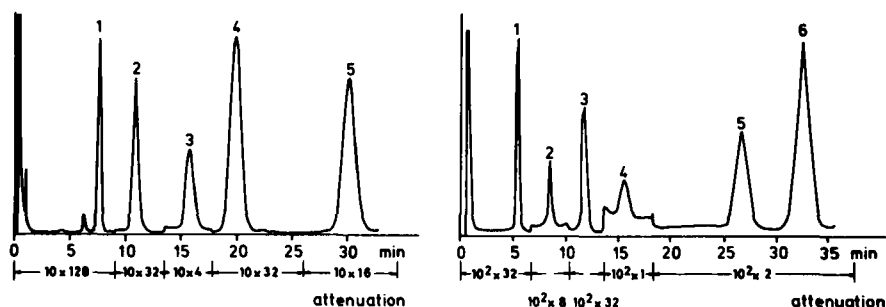


TABLE 20.6

ANALYSIS OF SIMULATED AND COMMERCIAL SUPPOSITORY PREPARATIONS⁴⁹

	Simulated preparation					Commercial suppository			
	1	2	3	4	5	2	6	4	5
Aminophenazone	300	299.8	6	1.01	-0.07	304.2	29	2.38	+1.40
d-Propoxyphene hydrochloride	60	60.4	6	0.67	+0.67	61.3	25	2.56	+2.17
Caffeine	50	50.5		1.62	+1.00	51.9		3.33	+3.80
Chlorpheniramine maleate	3	3.04		2.58	+1.33	3.08		5.16	+2.67
Lidocaine	20	20.2		2.40	+1.00	20.6		2.98	+3.00
Phenacetin	300	298.8	6	0.97	-0.40	299.3	20	2.20	-0.23
Aminophenazone	400	399.4		1.25	-0.15	397.7		2.61	-0.58
Caffeine	60	59.9		1.18	-0.17	59.5		3.53	-0.83
Chlorpheniramine maleate	4	3.99		2.72	-0.25	3.94		5.90	-1.50
Fenalamide	30	29.9		1.45	-0.33	29.6		3.46	-1.33
Sodium benzoate	60	59.6		1.32	-0.67	59.0		6.18	-1.67

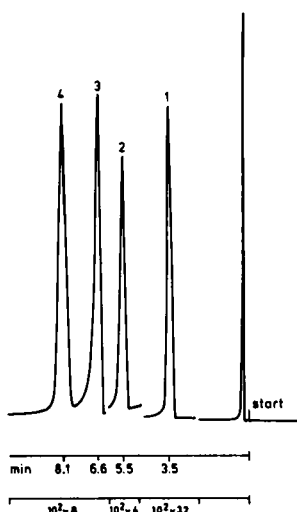
1 = weighed or declared amount in mg, 2 = found amount, average in mg, 3 = number of replicate analyses, 4 = relative standard deviation ($p = 0.05$), 5 = relative error, 6 = number of lots examined.

In order to run a series of analyses of drugs in pharmaceutical preparations, Fricke⁵⁰ made use of simple extractions. Tablets containing caffeine, phenacetin and aspirin were brought into a volumetric flask, to which chloroform and a small amount of glacial acetic acid were added. After shaking for 1 h chloroform was added and an aliquot was transferred to a Celite column that was treated with 1 N NaHCO_3 solution. After elution with chloroform, the eluate was evaporated to dryness, and the residue dissolved in methanol to about 0.3 mg/ml of caffeine. The gas chromatography was carried out on a packed column using Dexsil 300 10 % or 17 % on Chromosorb W HP at 220°C or 222°C respectively.

In a paper on the gas chromatographic assay of phenacetin, caffeine, antipyrin and dimethylaminoantipyrin in pharmaceutical preparations, Oesch and Sahli⁵¹ extracted the components by means of chloroform. After evaporation of the solvent the residue was redissolved in chloroform and gas chromatographed on a packed column of 2.5 % SE-30 on Chromosorb W HP at 180°C. A typical chromatogram is given in Figure 20.8.

FIGURE 20.8

CHROMATOGRAM OF PHENACETIN (1), CAFFEINE (2), ANTIPYRIN (3) AND DIMETHYLAMINOANTIPYRIN (4)⁵¹ on a packed column of 2.5 % SE-30 on Chromosorb W HP, 2 m long, at 180°C



De Vos and Jonkhoff⁵² described a gas chromatographic method to determine caffeine in several pharmaceutical preparations, *e.g.* tablets and suppositories. The tablet and suppository assay was carried out by dissolving or suspending the preparation in chloroform, to which the internal standard, lidocaine, had been added. The gas chromatographic separation was obtained on a 3 % OV-1 packed column at 160°C. Caffeine could be determined with a standard deviation of about 2 %.

20.3. THEOPHYLLINE

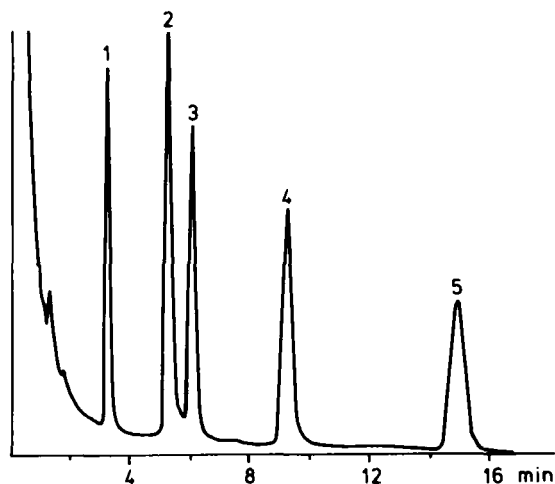
Because theobromine and theophylline have very low volatility and low solubility in most solvents, and are difficult to gas chromatograph without considerable adsorption losses, Brochmann-Hanssen and Oke¹⁶ preferred to convert both compounds into caffeine on flash-heater methylation by means of trimethylanilinium hydroxide. Caffeine can be readily gas chromatographed. To a screw-cap containing the substance to be gas chromatographed, trimethylanilinium hydroxide was added in approximately 100 % excess. The solution was diluted with methanol when necessary, and 1 μ l containing 0.2 to 1.0 μ g of the compound was injected into the flash heater with a micro syringe. The temperature of the flash heater was 275°C and the column temperature 137°C. A packed column of 3 % SE-30 on Gas Chrom Q was used.

In order to obtain better gas chromatographic properties for the quite polar xanthine derivatives, Kowblansky et al.¹⁹ introduced flash heater butylation with tetra-*n*-butylammonium hydroxide, using the commercially available titrant, a 25 % methanol solution (about 1 M). 7 μ l of the reagent were added to 1 ml methanolic xanthine solution containing not more than about 1.5 μ equiv. of N-H groups, at least a 4 : 1 molar ratio of alkylating agent to test compound. 1 μ l of the solution was injected into the gas chromatograph, using a minimum injector block temperature of 270°C. On a packed column of 3 % OV-17 on Gas Chrom Q and a column temperature of 220°C, very good separation of xanthines was obtained, as can be seen in the Figure 20.9.

FIGURE 20.9

CHROMATOGRAMS OF XANTHINES FOLLOWING FLASH HEATER N-BUTYLATION¹⁹

on a packed 3 % OV-17 column on Gas Chrom Q; injector block temperature 270°C, column temperature 220°C; 1 = caffeine, 2 = theophylline, 3 = theobromine, 4 = 1-methylxanthine, 5 = xanthine.



20.3.1. Theophylline in pharmaceutical preparations

Elefant et al.⁵³ were the first to use gas chromatography for the determination of theophylline in tablets that also contained other components (phenobarbital and ephedrine hydrochloride). One fine-ground tablet containing 130 mg theophylline was extracted with a chloroform-methanol (1 : 1) solution of the internal standard (4,4'-methylene-bis-(N,N-dimethylaniline) (0.6 mg/ml) and after centrifuging, the clear solution was injected into the gas chromatograph on a packed column with HI-EFF 8B 3 % on Gas Chrom Q at a column temperature of 250°C. Results of the assay are given in Table 20.7.

TABLE 20.7

ASSAY OF INDIVIDUAL TABLETS CONTAINING THEOPHYLLINE, EPHEDRINE AND PHENOBARBITAL⁵³
on a 3 % packed column of HI-EFF 8B on Gas Chrom Q at 250°C

Tablet	Ephedrine HCl	Phenobarbital	Theophylline
1	23.6	7.7	127.0
2	23.2	7.6	128.0
3	24.1	7.7	127.0
4	23.9	7.6	127.0
5	23.8	7.7	127.0
6	23.8	7.6	127.0
7	23.6	7.8	130.0
8	23.6	7.8	126.0
9	23.4	7.7	126.0
10	23.8	7.8	126.0
Average	23.7	7.7	127.0
Relative standard deviation	1.1 %	1.0 %	0.9 %

Schultz and Paveenbampen⁵⁴ determined theophylline in a suspension containing ephedrine and phenobarbital, using extraction, first of ephedrine at pH 11 with chloroform, and then theophylline and phenobarbital at pH 4.8 with the same solvent. Hexobarbital was used as an internal standard for the theophylline and phenobarbital determination. A recovery of 99.4 % and a coefficient of variation of ± 0.3 % was found for theophylline in a commercial suspension.

20.3.2. Theophylline in biological fluids

Theophylline is currently being used for the treatment of bronchial asthma and other cardiorespiratory disorders. Because there is good evidence that both the therapeutic response and the toxic side-effects are related to the concentration of the theophylline in the plasma, rather than to its dosage, accurate analytical methods are needed to make it possible to control treatment and reduce the risk of dangerous toxic symptoms.

Gas chromatographic methods have been developed to solve this problem. Theophylline has been gas chromatographed and determined as such, but in most cases derivatization has been carried out to obtain better gas chromatographic properties and better detection possibilities. For quantitative work several substances have been used as internal standard, mainly barbiturates (alphenal, amobarbital, aprobarbital, heptabarbital, hexabarbital, thiobarbital), but also other substances have been used (fluoranthene, codeine, theobromine, medazepam, pramoxine hydrochloride, cyheptamine, probenecid propylester). Recently 3-isobutyl-1-methyl

xanthine has been introduced as internal standard. In derivatization reactions this compound and theophylline are quite similar, as also in extractability, stability and chromatographic properties.

20.3.2.1. Determination of theophylline as such

A highly specific and reasonably sensitive method to determine theophylline in human serum was developed by Chrzanowski et al.⁵⁵. Samples of 3 ml serum were acidified with 0.1 N HCl and extracted with chloroform-isopropanol (95 : 5). After evaporation of the solvent, the residue was dissolved in 50 μ l of an internal standard solution containing 5 mg of Pramoxine hydrochloride in 50 ml chloroform-isopropanol mixture. About 1.3 μ l was injected onto the column of 3 % OV-17 on Chromosorb W at a column temperature of 240°C. Determination of theophylline in serum containing 4.9 μ g/ml to 10.1 μ g/ml could be carried out with a standard deviation of 0.4 μ g/ml to 0.3 μ g/ml, giving coefficients of variation of 8.0 % and 3.3 % respectively.

In a following paper, Chrzanowski et al.⁵⁶ stated that abnormal theophylline values obtained for blood serum samples that were kept in glass tubes sealed with butyl rubber stoppers, were caused by a substance which leached from the stoppers.

Wesley-Hadzija⁵⁷ worked out a simple method for the determination of theophylline in serum samples. To samples of 1 ml serum was added 0.5 ml 0.1 N HCl and the extraction was carried out with diethyl ether-dichloromethane (7 : 4), the organic solution evaporated to dryness, and the residue dissolved in 50 μ l methanol containing the internal standard (500 μ g/ml of codeine). An aliquot of 2-5 μ l was gas chromatographed on a packed column of 3 % OV-17 on Gas Chrom Q at a column temperature of 245°C. The reproducibility of the procedure was good.

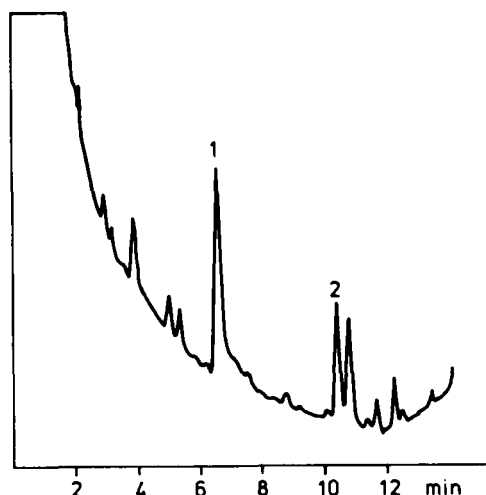
Because most gas chromatographic methods for the determination of theophylline in biological fluids involve tedious extraction procedures and/or derivatization prior to the gas chromatographic analysis, Sheehan and Haythorn⁵⁸ developed a rapid method for such determinations. Theophylline was extracted from acidified blood with chloroform after the addition of the internal standard, cyheptamide, and it was gas chromatographed directly on a packed column of 3 % OV-1 on Chromosorb W using temperature programming from 160°C to 280°C. Concentrations down to 2 μ g/ml blood could be determined, with recoveries ranging from 90 to 110 %. A typical chromatogram is given in Figure 20.10.

An interesting technique for gas chromatographic determination of theophylline in dried whole blood was developed by Albani and Toseland⁵⁹. About 35 mg whole blood was applied on filter paper cards by capillary blood sampling techniques. 3 μ l of the internal standard solution (heptabarbital) were added to the spot. Three to six spots were punched out and were extracted with 500 μ l of phosphate buffer pH 4.5 and 5 ml of distilled diethyl ether. After evaporation of the diethyl ether, the residue was dissolved in 15 μ l of methanol. 2 μ l were injected in the gas chromatograph, which was equipped with a nitrogen specific detector and a packed column of 0.5 % HI-EFF 8B on Chromosorb W HP. Column temperature was 240°C. Analysis of ten samples, with identical concentration, on ten successive days, revealed a day-to-day variance of ± 2.9 %; the precision of the method, as studied by analysis of ten different samples with identical concentrations, revealed a coefficient of variation of 5.2 % (range 6.7 % to 3.8 %) in five subsequent investigations.

FIGURE 20.10

CHROMATOGRAM OF AN EXTRACT FROM 2 ML BLOOD CONTAINING 40 μ g OF THEOPHYLLINE⁵⁸

on a 3 % packed column of OV-1 on Chromosorb W and temperature programming from 160 to 280°C
1 = theophylline, 2 = cyheptamide (internal standard).



Chambers⁶⁰ described a rapid method for the determination of underivatized theophylline in plasma. To a 500 μ l sample of plasma was added the internal standard, heptabarbital, then the sample was acidified and extracted with chloroform. The residue after evaporation of the chloroform was dissolved in 10 μ l acetone and 4 μ l was gas chromatographed on a 3 % poly (cyclohexyldimethanol) succinate on Diatomite column at 225°C. A chromatogram of an extract of the plasma is given in Figure 20.11.

Both Albani and Toseland⁵⁹ and Chambers⁶⁰ made use of an organic-nitrogen specific (alkali-flame ionization) detector.

20.3.2.2 Determination of theophylline after derivatization

A. Packed columns

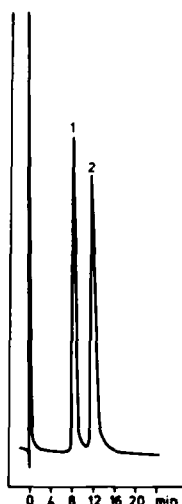
I. Propylation

Shah and Riegelman²⁷ extracted theophylline from plasma and/or saliva samples (1 ml) with a mixture of diethyl ether, dichloromethane and isopropanol (6 : 4 : 1). Theophylline was removed from the organic solution by means of aqueous sodium hydroxide, the alkaline solution was acidified with phosphoric acid (pH 5) and theophylline was re-extracted with the organic mixture mentioned. The internal standard (thiobarbital or fluoranthene) was added, the solvent evaporated, and the residue dissolved in 25 μ l of tetrapropylammonium hydroxide and gas chromatographed on a packed column of 3 % OV-17 on Gas Chrom Q at 190°C. In the injection heater (kept at 265°C) theophylline is quantitatively converted to its propyl derivative, which gives a symmetrical peak distinct from any other xanthines, or barbiturates.

FIGURE 20.11

CHROMATOGRAM OF AN EXTRACT OF PLASMA CONTAINING THEOPHYLLINE⁶⁰

on a 3 % poly(cyclohexyldimethanol) succinate on Diatomite packed column at 225°C; 1 = heptabarbital (internal standard, 160 μ mole/l; 2 = theophylline, 95 μ mole/l



Zuidema et al.⁶¹ used Propyl-8 (= dimethylformamide-dipropylacetal) for propylation of theophylline. Fluoranthene was used as an internal standard for the determination of theophylline in biological fluids. Extraction was obtained with chloroform-isopropanol (95 : 5) after acidifying the sample (1 ml) with hydrochloric acid. After evaporation of the solvent the residue was dissolved in 0.5 ml of a methanolic solution of the internal standard. The methanol was evaporated and the residue dissolved in 30 μ l Propyl-8 and gas chromatographed on a packed column of 3 % OV-17 on Gas Chrom Q at a column temperature of 220°C

II. Methylation

In a determination of purine and pyrimidine base ratios in nucleic acids and oligonucleotides, Mac Gee¹⁵ methylated them in the flash heater of the gas chromatograph by thermal decomposition of their tetramethylammonium salts. Theophylline and theobromine (50 μ moles) were dissolved in 0.5 ml of 1 M tetramethylammonium hydroxide in ethanol. While the free purines are not very soluble in ethanol, their tetramethylammonium salts are quite soluble. The gas chromatography was performed on a 6 feet by 4 mm stainless steel column packed with 80-100 mesh glass beads coated with 0.5 % Carbowax 20 M. Column temperature was 143°C and flash heater temperature 360°C. With complete methylation, theophylline and theobromine yielded caffeine.

Because the peak shape of theophylline can be poor in gas chromatography, as also can be the sensitivity of the method, Dusci et al.¹⁷ preferred to convert theophylline with on-column methylation by means of trimethylanilinium hydroxide to caffeine. In that manner they

developed a method for the assay of theophylline in plasma that was sensitive down to 0.1 mg% theophylline. A similar extraction and purification procedure was proposed by Shah and Riegelman²⁷. A packed column of 3 % OV-225 on Chromosorb W was used and the column temperature was 235°C. Recovery was for amounts of 6-48 µg theophylline added to plasma samples (2 ml) 88 ± 8 %. Internal standard was medazepam.

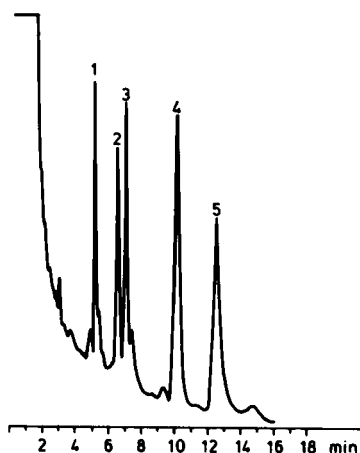
Kinsun et al.¹⁸ used on-column methylation, but trimethylammonium hydroxide was used as the methylating agent. A single extraction of serum samples (50 µl) with chloroform-diethyl ether (6 : 4) was carried out and theophylline could be determined by the method down to 0.5 µg/ml. A nitrogen selective detector was used, with heptabarbital as internal standard. The gas chromatographic column was a packed one with 3 % OV-1 on Gas Chrom Q operating at 210°C.

III. Butylation

For the determination of theophylline and probenecid in biological material, Arbin and Edlund²⁰ used dimethylformamide di-*n*-butyl acetal as the butylating agent. The samples (0.2-1.0 ml) were extracted with chloroform over a cellulose column treated with phosphate buffer pH 7. The eluate containing theophylline and probenecid was evaporated to dryness and the residue was dissolved in 60 µl internal standard solution (probenecid propylester). After the addition of 40 µl of the butylating agent, the mixture was kept at room temperature for five minutes and then 1 µl was injected into the gas chromatograph. A packed column of 3 % OV-17 on Chromosorb W and temperature programming from 190°C to 240°C was used. A chromatogram is given in Figure 20.12.

FIGURE 20.12

CHROMATOGRAM OF CAFFEINE (1), THE *n*-BUTYL DERIVATIVES OF THEOPHYLLINE (2), THEOBROMINE (3) AND PROBENECID (5) AND PROBENECID PROPYLESTER (4) (= INTERNAL STANDARD)²⁰ on a 3 % OV-17 packed column on Chromosorb W with temperature programming, 190-240°C



The extraction procedure and the derivatization give the method high selectivity. Quantitative determinations down to 2 μg theophylline and 4 μg probenecid can be done. The addition of 5 μg theophylline to a sample gave an absolute recovery of $91 \pm 5\%$.

Johnson et al.²¹ made use of the butylation of theophylline in connection with its quantitative determination in human serum and saliva. The butylation described by Kowblansky et al.¹⁹, whereby mixtures of caffeine, theobromine and theophylline can easily be separated on a polar phase such as SP 2250 and OV-17, requires quite a high injector temperature (300°C) to obtain optimum alkylation. The butylation described by Greely⁶², which is an off-column butylation by means of butyl iodide, avoids the pyrolytic production of trialkylamine that occurs when a tetraalkylammonium hydroxide is injected, and this reaction is therefore preferable for the analysis of xanthines. Aprobarbital and alphenal were used as internal standards. A packed column with 3 % SP 2250 on Supelcoport and temperature programming from 160°C to 240°C was used.

Perrier and Lear²⁴ converted theophylline to its butyl derivative by means of tetrabutylammonium hydroxide and on-column alkylation in connection with a rapid extraction procedure. Samples of 100 μl plasma were extracted with chloroform-isopropanol (95 : 5) containing the internal standard, amobarbital. The solvent was evaporated and the residue dissolved in 1 ml toluene. With the aid of 10 μl of an aqueous solution of tetrabutylammonium hydroxide, theophylline and amobarbital were quantitatively extracted from the toluene, and by injection of an aliquot of this solution into the gas chromatograph, alkylation took place in the injector, and quantitation of theophylline in concentrations in 100 μl plasma was possible with a flame ionization detector, using a packed column with 3 % OV-17 on Chromosorb G at 250°C .

Least et al.²³ reduced the sample size to 20 μl serum, plasma, or saliva, in a micro-scale procedure. The extraction was carried out with a chloroform-isopropanol solution of the internal standard, 3-isobutyl-1-methylxanthine. After alkylation with tetramethylammonium hydroxide and pentyl iodide, gas chromatography was carried out on a packed 3 % OV-17 on Gas Chrom Q column, by temperature programming from 180°C to 260°C , and using a nitrogen sensitive detector. With the sample volume used, the background interference is equivalent to about 0.1 mg/litre, and 0.5 mg theophylline per litre can easily be measured. Between-run precision was 2.8 % by theophylline concentrations of 14.8 mg/litre.

To improve the theophylline analysis, Bailey et al.²² used 3-isobutyl-1-methylxanthine as an internal standard. This substance had already been used by Dechtiaruk et al.²⁸, Schwertner et al.³¹ and Least et al.²³. Extraction was performed with serum samples of 2 ml with a chloroform-isopropanol solution (95 : 5) of the internal standard. After purification via extraction into aqueous sodium hydroxide, and back extraction into chloroform-isopropanol, the sample was derivatized with *n*-butyl-8-reagent. No interference was encountered from normal serum constituents or methylxanthines. Recovery was 85 %, and precision and accuracy were good. An 10 % SE-30 on Chromosorb W column at 200°C was used.

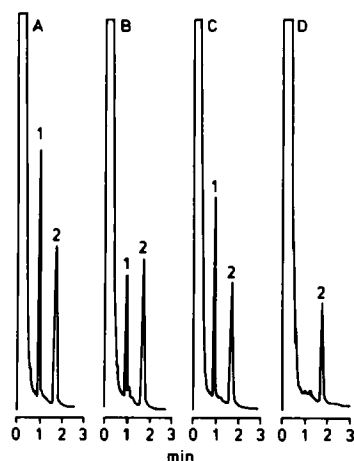
Pranskevich et al.²⁵ preferred a packed column of 3 % SP 2250 DB as stationary phase because the butyl derivative, which is a weak base, gas chromatographs markedly better on this phase than on the same percentage loadings of other brands of OV-17. After an initial chloroform extraction of 1.0 ml serum sample, a toluene wash and back extraction into 0.5 M ammonium hydroxide, derivatization was accomplished by the addition of 15 μl of an 8 : 1 mixture of *N,N*-dimethylacetamide (400 μl) and 2.4 % tetramethylammonium hydroxide in methanol (50 μl) to the dried extract. Butyl iodide (15 μl) was then added and the gas chromatographic assay

carried out. The stationary phase SP 2250 DB was ideal for the analysis since an alkaline tetramethylammonium hydroxide solution was used. Typical gas chromatograms are given in Figure 20.13.

FIGURE 20.13

GAS CHROMATOGRAMS OF THEOPHYLLINE²⁵

Column: Glass, 90 cm by 2 mm I.D., packed with 3 % SP 2250-DB on Supelcoport, temp. 200°C. (A) standard-theophylline 20 µg/ml and internal standard (heptabarbital) 10 µg/ml; (B) control serum-theophylline 10 µg/ml and internal standard 10 µg/ml; patient-serum specimen (C) from patient receiving theophylline and internal standard 10 µg/ml; (D) blank-serum and internal standard 10 µg/ml. (1) = theophylline, (2) = internal standard.



A seven minutes determination of theophylline in less than 100 µl of serum was described by Vinet and Zizian²⁶. To 100 µl of serum was added 50 µl phosphate buffer pH 4.2, 50 µl methanol and 200 µl of a mixture of diethyl ether, dichloromethane and isopropanol (6 : 4 : 1). After shaking, 1.6 µl of the extract plus 0.4 µl of a mixture of butyliodide, methanol and tetrabutylammonium hydroxide, was injected into the gas chromatograph using a 3 % OV-17, 0.1 % terephthalic acid on Chromosorb 750 at a column temperature of 230°C. As internal standard 3-isobutyl-1-methylxanthine was used, with a sensitive nitrogen detector. The results correlated well with those obtained by ultraviolet spectrometry.

IV. Pentylation

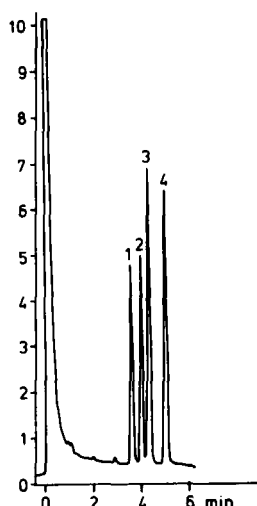
The method described by Johnson et al.²¹, whereby off-column butylation by means of butyl iodide was used for the butylation, was improved by Dechtiaruk et al.²⁸ by using 3-isobutyl-1-methylxanthine as internal standard and pentyliodide as alkylating agent. Pentyl iodide gave optimal resolution between the derivatives of the compounds of interest. Figure 20.14 shows the separation of the pentyl derivatives of theophylline, theobromine, phenobarbital and the internal standard.

To obtain greater sensitivity and greater selectivity in the analysis of serum theophyl-

FIGURE 20.14

PENTYL DERIVATIVES OF THEOPHYLLINE (1), THEOBROMINE (2), 3-ISOBUTYL-1-METHYLYXANTHINE (3) AND PHENOBARBITAL (4)²⁸

on a packed column of 3 % OV-17 on Gas Chrom Q, with temperature programming, 190-240°C



line, Lowry et al.²⁹ used a nitrogen sensitive detector and 3-isobutyl-1-methylxanthine as an internal standard. The method involved a single extraction of 50 μ l serum, and the derivatization of theophylline and the internal standard to their pentyl derivatives was performed by dissolving the compounds in N,N-dimethylacetamide, and adding trimethylammonium hydroxide and propyliodide. A packed column with 3 % OV-17 on Gas Chrom Q was used at a column temperature of 240°C. Using 50 μ l of serum, concentrations of 1 μ g/ml in serum could easily be measured. Precision of the method was 3.4 % for therapeutic doses of theophylline.

V. Pentafluorobenzoylation

To be able to determine theophylline in the ng range, Arbin and Edlund³⁰ improved their method²⁰ by derivatizing theophylline and the internal standard theobromine using pentafluorobenzyl bromide, in connection with an electron capture detector. To 100 μ l samples of plasma containing 50-1000 ng theophylline, 100 μ l of a solution of the internal standard were added. The extraction was performed by column extraction (cellulose) using dichloromethane as solvent. After back extraction into an alkaline aqueous phase (NaOH), theophylline and theobromine were alkylated with pentafluorobenzyl bromide by an extractive alkylation technique. The derivatives were extracted with cyclohexane and, after concentration to 3 ml, 2 μ l were injected for the gas chromatographic assay. Standard deviation of the method was 2.5 % at a concentration level of 200 ng per sample, and 8 % at 20 ng per sample. The sample amounts were 100 μ l plasma and 250 mg rat brain tissue, and the sensitivity limit

about 5 ng per sample. A packed column with 3 % XE-60 on Gas Chrom Q at 220⁰ was used for the gas chromatographic assay.

Schwertner et al.³¹ introduced a salting-out procedure, in combination with single extraction using chloroform-isopropanol (95 : 5). 100 μ l plasma samples could be effectively extracted with 2 ml chloroform-isopropanol. Theophylline was derivatized with pentafluorobenzyl chloride, and 3-isobutyl-1-methylxanthine was used as an internal standard. This standard is similar to theophylline in extractability, derivatization rates, stability and chromatographic properties. Accurate measurements of plasma concentrations (\pm 0.22 μ g/ml) could be obtained with little or no interference from theophylline metabolites and other coextractable material. A packed column with 3 % OV-17 on Gas Chrom Q and temperature programming from 150⁰C to 250⁰C was used in combination with an electron capture detector.

B. Capillary columns

I. Ethylation

Glass capillary gas chromatography was applied by Floberg et al.⁶³ for the simultaneous determination of theophylline and caffeine in small volumes (50 μ l) of plasma from premature infants, after the samples' extraction as ion-pairs with tetramethylammonium into dichloromethane and acylation with ethyl iodide - followed by GC-MS. The gas chromatography was performed on a 25 m OV-225 glass capillary column and temperature programming (170-210⁰C - 10⁰C per min) using trideuterotheophylline and hexadeuterocaffeine as internal standards. The compounds could also be separated as pentafluorobenzyl derivatives on a 25 m OV-17 glass capillary. The precision of the method was 3.9 % for theophylline and 3.2 % for caffeine (0.6 μ g per ml plasma, n = 10) and the detection limit was 20 and 40 ng/ml plasma for theophylline and caffeine, respectively.

TABLE 20.8.

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF XANTHINE ALKALOIDS

Column	Solid support mesh	Stat. phase	%	Temperature	Comp. Prep.	Ref.
glass, 6 ft x 4 mm I.D.	CW 80-100	SE-30	2-3	204 ⁰ C	alk.s.	1
glass, 6 ft x 3 mm I.D.	GP 100-140	SE-30	1.15	175 ⁰ C	alk.s.	2
s.s., 5 ft x 2.4 mm I.D.	C AW 60-80	SE-30	5	210 ⁰ C	alk.s.id.tox.	3
glass, 6 ft x 4 mm I.D.	Ana ABS 100-120	SE-30	1	150-180 ⁰ C	alk.s.id.tox.	4
-	-	QF-1	3	200 ⁰ C		
glass, 1 m x 2 mm I.D.	CW S 80-100	NPGS	1	200 ⁰ C	alk.s.	5
glass, 3 ft x 0.07 in I.D.	GP AS 80-100	SE-30	1	175 ⁰ C		
-	-	XE-60	1	220 ⁰ C	alk.s.	6
-	-	EGSS-Y	1	230 ⁰ C		
-	-	HI-EFF	8B1	230-240 ⁰ C		
-	GQ S 100-120	NGS	1	230 ⁰ C		7
-	GP 80-100	PVP	1	200 ⁰ C		
-	-	+ NGS	1			
glass, 3 ft x 1/8 in O.D.	GP S 100-120	HI-EFF	8B1	220-230 ⁰ C	alk.s.id.tox.	8
6 ft x 1/4 in	CW 60-80	SE-30	1.5	200-300 ⁰ C	pr. xths.s.	9
s.s.S, 1 m x 3 mm I.D.	CW AWS 80-100	SE-30	1.5	190 ⁰ C		
-	-	SE-30	3	200 ⁰ C		
-	-	SE-52	1.5	210 ⁰ C	xths., tp.TMS.TFA.	
-	-	QF-1	3	210 ⁰ C	der.s.id.MS	10
-	-	OV-17	2	240 ⁰ C		
-	-	XE-60	3	230 ⁰ C		

TABLE 20.8 (continued)

Column	Solid support mesh	Stat.phase	%	Temperature	Comp.Preparation	Ref.
s.s. cap., 100 ft x 0.01 in I.D.		QF-1		100-200°C	pr.	
- 200 ft x 0.01 in I.D.		SE-30		100-250°C	pr. ca.	11
- 100 ft x 0.01 in I.D.		Apiezon L		100-250°C	pr.	
glass cap., 12 m x 0.3 mm I.D.		Triton X 305		200-250°C	pr. ca. her.smpl.	12
glass cap., 20 m x 0.35 mm I.D.		SE-30		50-250°C	pr. ca.	13
glass cap., 12 m x 0.49 mm I.D.		CP-Sil 5				
f.sil. cap., 12 m x 0.22 mm I.D.		CP-Sil 5			drg.ca.s.id.	14
s.s., 5 ft x 1/8 in O.D.	GQ 100-120	SE-30	3	137°C	tb.tp.Me.der.	16
glass S, 6 ft x 4 mm I.D.	CW HP 100-120	OV-225	3	235°C	tp.Me.der.	17
s.s., 2 m x 2.37 mm	GQ 100-120	OV-1	3	210°C	tp.Me.der.	18
	GQ	OV-17	3	220°C	xths.Bu.der.	19
glass S, 180 cm x 3 mm I.D.	CW 80-100	OV-17	3	190-240°C	pr. tp.Bu.der.	20
glass, 122 cm x 2 mm I.D.	Sup. 100-120	SP 2250	3	160-240°C	pr. tp.Bu.der.qnt.	21
glass S, 5 ft x 0.25 in O.D.	CW HP 100-120	SE-30	10	220°C	tp.Bu.der.qnt.	22
glass, 91 cm x 2 mm I.D.	GQ 100-120	OV-17	3	180-260°C	pr. tp.qnt.pl.	23
glass, 1.8 m x 2 mm I.D.	CG 100-120	OV-17	3	250°C	tp.Bu.der.qnt.	24
glass, 90 cm x 2 mm I.D.	Sup.100-120	SP 2250-	3	200°C	tp.Bu.der.qnt.	25
glass, 1.8 m x 2 mm I.D.	C 750 100-120	OV-17	3	230°C	tp.Bu.der.qnt.	26
		+ thra.	0.1		pl.	
glass, 1.83 m x 3.1 mm	GQ	OV-17	3	190°C	tp.Pr.qnt.bi.	27
glass, 122 cm x 2 mm I.D.	GQ 100-120	OV-17	3	190-240°C	pr. tp.Pe.der.qnt.	28
glass, 6 ft x 2 mm I.D.	GQ 100-120	OV-17	3	240°C	tp.Pe.der.ser.	29
glass S, 180 cm x 3 mm I.D.	GQ 80-100	XE-60	3	220°C	tp.PFbz.der.	30
glass, 1 m x 4 mm I.D.	GQ 100-120	OV-17	3	150-250°C	pr. tp.PFbz.qnt.pl.	31
2 m	MGB	Cab 20M	0.2	210°C	ca.qnt.coex.	32
glass, 6 ft x 4 mm I.D.	GQ 80-100	DC 200	10	190°C	ca.qnt.tea	33
glass, 5 ft x 1/4 in	Phs.N 60-85 AWS	Ver.930	2	200°C	ca.qnt.co	35
glass, 5 ft x 3 mm I.D.	Var.30 70-80	SE-30	10	215°C	ca.qnt.co	36
s.s., 80 cm x 1/8 in	CG HP AWS 100-120	Cab 20M	2	220°C	ca.qnt.co	37
glass, 90 cm x 2 mm I.D.	CG AWS 80-100	OV-17	2.5	260°C	ca.qnt.dr.	38
s.s., 6 ft x 1/8 in O.D.	CW HP AWS 100-120	OV-17	3	200°C	ca.qnt.pl.	39
glass, 5 ft x 0.25 in O.D.	CQ 80-100	Dex 300	3	210°C	ca.qnt.bl.	40
glass, 182 cm x 2.5 mm I.D.	CP 60-80	OV-17	3	210°C	ca.qnt.pl.	41
glass, 180 cm x 2 mm I.D.	CW AWS 80-100	SE-30	3	190°C	ca.qnt.pl.	42
glass, 1.5 m x 4 mm I.D.	Srb 60-80	Apiezon M	10	225°C		
glass, 2.4 m x 4 mm I.D.	GQ S 60-80	Poly S-179	3	225°C	ca.qnt.pl.	44
s.s., 6 ft x 4 mm I.D.	Hal.F 60-80	DC 200	2	75-200°C	pr. ca.qnt.pre.	45
s.s., 1.8 m x 2 mm	Cel. 80-100	SE-30	10	215°C	ca.qnt.pre.	46
s.s., 6 ft x 1/8 in O.D.	CW AW	SE-30	10	195-260°C	pr. ca.qnt.pre.	47
glass, 4 ft x 4 mm I.D.	GQ 100-120	OV-17	3	165°C	ca.qnt.pre.	48
glass, 2 m x 4 mm I.D.	GP + KOH 5 %	Cab 20M	2	200°C		
		+ SE-30	2			
glass, 2 m x 4 mm I.D.	Dia.S 80-100	UCC W98	10	205°C	ca.qnt.sup.	49
glass S, 6 ft x 4 mm I.D.	CW HP 80-100	Dex 300	10	220°C		
- 8 ft x 4 mm I.D.	CW HP 80-100	Dex 300	17	222°C	ca.qnt.pre.	50
glass, 2 m	CW HP 80-100	SE-30	2.5	180°C	ca.qnt.pre.	51
glass, 150 cm x 3 mm I.D.	CW HP 80-100	OV-1	3	160°C	ca.qnt.pre.	52
glass, 6 ft x 0.25 in O.D.	GQ 100-120	HI-EFF 8B	3	250°C	tp.tb.qnt.	53
glass, 1.8 m x 4 mm I.D.	GQ 100-120	OV-17	3	200°C	tp.qnt.pre.	54
glass S, 6 ft x 1/8 in I.D.	CW HP 100-120	OV-17	3	240°C	tp.qnt.ser.	55,56
glass, 6 ft x 1/4 in O.D.	GQ 100-120	OV-17	3	245°C	tp.qnt.ser.	57
glass, 1.83 m x 2 mm I.D.	CW HP 80-100	OV-1	3	180-280°C	pr. tp.qnt.bl.	58
glass, 4 ft x 2 mm I.D.	CW HP 80-100	HI-EFF 8B	0.5	240°C	tp.qnt.bl.	59
glass, 1 m x 4 mm I.D.	Diat CLQ	PCHDS	3	225°C	tp.qnt.pl.	60
2 m x 3 mm	GQ 100-120	OV-17	3	190-220°C	tp.Pr.der.qnt.	61
					bi.	

TABLE 20.8 (continued)

Column	Solid support	Stat.phase %	Temperature	Comp. Prep.	Ref.
glass cap., 25 m		OV-225	170-210°C	pr. tp.Eth.der.qnt.	63
-		OV-17	170-210°C	pr. pl.	

TABLE 20.9

XANTHINE ALKALOIDS - LIST OF ABBREVIATIONS

ABS = acid, base washed, silanized	Me = methyl
alk = alkaloid	MGB = micro glass beads
Ana = Anakrom	NGS = neopentyl glycol succinate
AW = acid washed	NPGS = neopentyl glycol sebacate
bi = biological material	O.D. = outside diameter
bl = blood	PCHDS = poly(cyclohexyldimethanol succinate)
Bu = butyl	Pe = pentyl
C = Chromosorb	PFbz = pentafluorobenzyl
ca = caffeine	Phs.N = Phasesep N
cap = capillary	pl = plasma
Cel = Celite	pr = (temperature) programming
co = coffee	Pr = propyl
coex = coffee extract	pre = pharmaceutical preparation
CP = Chromosorb P	qnt = quantitative
CW = Chromosorb W	s = separation
Dia = Diatoport	S = silanized
Diat = Diatomite	ser = serum
der = derivative	smpl = sample
Dex = Dexsil	Srb = Supasorb
dr = drink	s.s. = stainless steel
drg = drug	sup = suppository
Eth = ethyl	Sup = Supelcoport
f.sil = fused silica	tb = theobromine
GP = Gas Chrom P	TFA = trifluoroacetyl
GQ = Gas Chrom Q	thra = therephthalic acid
Hal = Haloport	TMS = trimethylsilyl
her = heroin	tox = toxicology
HP = high performance	tp = theophylline
I.D. = inside diameter	Var = Varaport
id = identification	xths = xanthines
in = inch	

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II.8 MISCELLANEOUS ALKALOIDS

Chapter 21

CEPHALOTAXUS ALKALOIDS

21.1. <i>Cephalotaxus</i> alkaloids	213
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21.1. CEPHALOTAXUS ALKALOIDS

Many alkaloids of the genus *Cephalotaxus* have demonstrated antitumour activity. Spencer et al.¹ applied gas chromatography to separate the components of crude alkaloid mixtures of *Cephalotaxus* species. By combined GC-MS, the identity of known alkaloids could be confirmed and the presence of unknown alkaloids demonstrated. Estimation of the various alkaloids was obtained by using methyl lignocerate ($C_{24:0}$) as an internal standard by gas chromatography on a packed column of 3 % Dexsil 300 on Gas Chrom Q and temperature programming from 180°C to 285°C. Gas chromatograms of standards and of a plant extract are found in Figure 21.1, the chromatographic data in Table 21.1 and the formulas of the alkaloids in Figure 21.2. The results obtained by the assay of *Cephalotaxus* alkaloid extracts of various species are listed in Table 21.2.

FIGURE 21.1

CHROMATOGRAMS OF STANDARDS (A) AND A PLANT EXTRACT (B)¹.

1 = Cephalotaxine (Ia), 2 = homoerythrina alkaloids (VIa and b and VIIa and b), 3 = cephalotaxinone artefact, 4 = drupacine (II), 5 = 11-hydroxycephalotaxine (III), 6 = homoerythrina alkaloids (VIIa and b), 7 = internal standard ($C_{24:0}$), 8 = cephalotaxinone (IV), 9 = unidentified alkaloid, 10 = deoxyharringtonine (Ie), 11 = isoharringtonine (Id), 12 = harringtonine (Ib), 13 = homoharringtonine (Ic).

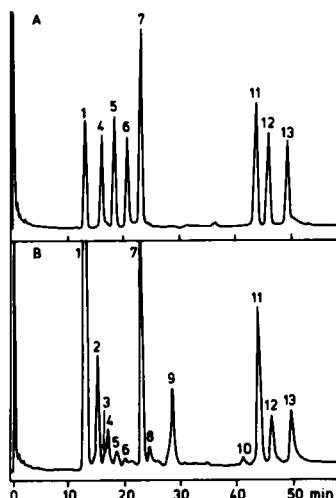


TABLE 21.1

CHROMATOGRAPHIC DATA FOR *CEPHALOTAXUS* ALKALOIDS¹

Peak numbers refer to Figure 21.1.

Peak No.	Compound	t _R (Rel)
1	Cephalotaxine	0.55
2	Homoerythrina alkaloids (VIa and b, VIIa and b and IX)	0.67
3	Cephalotaxinone artefact	0.71
4	Drupacine (II)	0.73
5	Acetylcephalotaxine (If)	0.79
	11-Hydroxycephalotaxine (III)	0.80
	Desmethylcephalotaxinone (V)	0.80
6	Homoerythrina alkaloids (VIIIa and b)	0.88
7	Internal standard (C ₂₄ :0)	1.00
8	Cephalotaxinone (IV)	1.05
9	Unidentified alkaloid	1.24
10	Deoxyharringtonine (Ie)	1.76
11	Isoharringtonine (Id)	1.90
12	Harringtonine (Ib)	2.00
13	Homoharringtonine (Ic)	2.14

TABLE 21.2

GAS CHROMATOGRAPHIC ANALYSIS OF SELECTED *CEPHALOTAXUS* ALKALOID EXTRACTS¹

Species	Plant part	Percentage of total alkaloids							
		Ia	Ib	Ic	Id	Ie	II	III	VIIIa and b
<i>harringtonia</i> var. <i>drupacea</i>	Seed	33	8.3	1.2	3.9	0.7	35	9.6	6.5
<i>harringtonia</i> var. <i>harringtonia</i>	Root	39	6.6	7.5	19	2.4	3.7	1.1	3.4
<i>harringtonia</i> var. <i>harringtonia</i>	Leaf	32	2.4	4.1	13	1.3	5.3	2.6	2.9
<i>harringtonia</i> var. <i>harringtonia</i>	Whole plant	45	3.7	8.5	20	1.5	3.7	2.1	2.2
<i>fortunei</i>	Leaf	64	0.3	0.1	0.3	-	6.8	1.4	4.2
<i>fortunei</i>	Seed	52	4.4	tr.	0.9	0.4	7.0	2.2	4.6
<i>wilsoniana</i>	Seed	29	6.1	0.2	0.4	-	0.7	0.9	45
<i>griffithii</i>	Root-leaf	40	0.9	1.5	11	4.3	9.0	2.5	1.7

tr. = trace

TABLE 21.3

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF *CEPHALOTAXUS* ALKALOIDS

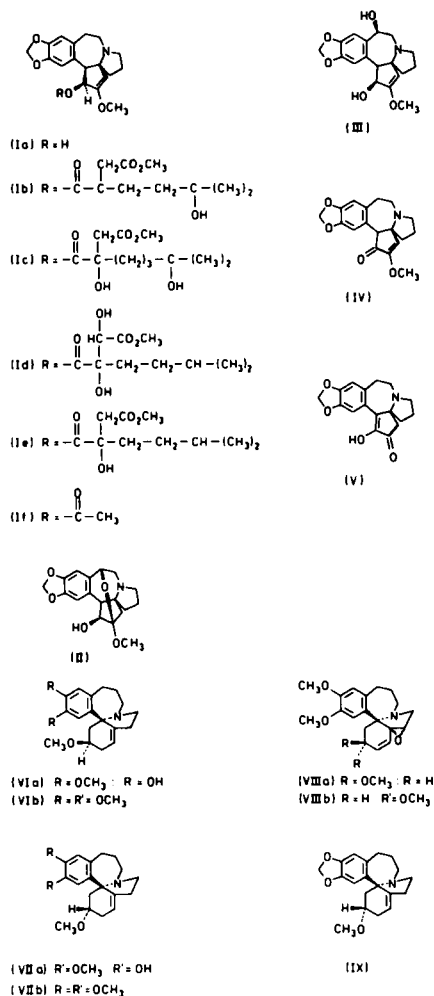
Column	Solid support mesh	Stat.phase	%	Temperature	Comp.Preparation	Ref.
glass, 4 ft	GQ 100-120	Dex. 300	3	180-285°C	pr. alk.s.	1

Abbreviations: Dex. 300 = Dextral 300, pr. = (temperature) programming, alk. = alkaloid, s = separation

FIGURE 21.2

CEPHALOTAXUS ALKALOIDS¹

Numbers refer to Figure 21.1 and Table 21.1.



21.2 REFERENCES

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Chapter 22

IMIDAZOLE ALKALOIDS

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22.1 PILOCARPINE

Pilocarpine was gas chromatographed by Brochmann-Hanssen and Baerheim Svendsen¹ on a packed column of 1.15 % SE-30 at 175°C. Brochmann-Hanssen and Fontan^{2,3} chromatographed pilocarpine on packed columns of various polarity, *e.g.* XE-60, EGSS-Y, HI-EFF 8 B, as well as NGS and PVP + NGS. Massingill and Hodgkins⁴ used packed columns of various polarities and they separated pilocarpine and isopilocarpine on 1% JXR and 0.5 % Epon 1001 Resin with retention times of 9.08 min and 8.75 min, respectively, on JXR, and 11.50 min and 11.67 min, respectively, on Epon 1001 Resin.

Bayne *et al.*⁵ developed a method for the assay of sub-nanograms of pilocarpine in biological fluids. Because a significant tailing was observed when pilocarpine and isopilocarpine were gas chromatographed, as well as a tendency for pilocarpine to epimerize thermally to isopilocarpine, the authors acylated the imidazole ring with heptafluorobutyric anhydride, using triethylamine as a catalyst. The pilocarpine base was extracted with dichloromethane from an aqueous solution after adjustment of the pH to 9. It was subjected to derivatization and a clean-up procedure, and gas chromatographed on a packed 3 % OV-17 on Chromosorb W column at 190°C. The method described is specific for pilocarpine, with the isopilocarpine derivative eluting prior to the pilocarpine derivative. The limit of sensitivity was 25-50 pg of pilocarpine using EC-detection. Methazolamide was used as an internal standard - also after derivatization - to dimethylmethazolamide. Typical gas chromatograms are found in Figure 22.1.

TABLE 22.1

EXPERIMENTAL CONDITIONS USED FOR GAS CHROMATOGRAPHY OF PILOCARPINE

Column	Solid support mesh	Stat.phase	%	Temperature	Comp. Prep.	Ref.
glass, 6 ft x 3 mm I.D.	GP 100-140	SE-30	1.15	175°C	alk.s.	1
glass, 3 ft x 2 mm I.D.	GP AWS 80-100	SE-30	1	175°C		
-	-	XE-60	1	220°C	alk.s.	2
-	-	EGSS-Y	1	230°C		
-	-	HI-EFF 8B	1	230°C		
glass, 3 ft x 2 mm I.D.	GP AWS 80-100	NGS	1	230°C	alk.s.	3
-	GP 80-100	PVP	1	200°C		
		+ NGS	1			
copper, 6 ft x 1/8 in O.D.	GP 100-120	JXR	1	100-300°C pr.	alk.s.	4
copper, 2 ft x 1/8 in O.D. Dia S	80-100	Epon	0.5	100-250°C pr.		
glass S, 1.8 m x 2 mm I.D.	CW 100-120	OV-17	3	190°C	pil.der.qnt.bi.	5

FIGURE 22.1

CHROMATOGRAMS OF PILOCARPINE DERIVATIVE⁵

prepared from aqueous pilocarpine nitrate solution carried through extraction, derivatization and clean-up procedure. A = internal standard, methazolamide derivative; B = pilocarpine derivative; I = pilocarpine base equivalent to 50 pg; II = pilocarpine base equivalent to 1 ng.

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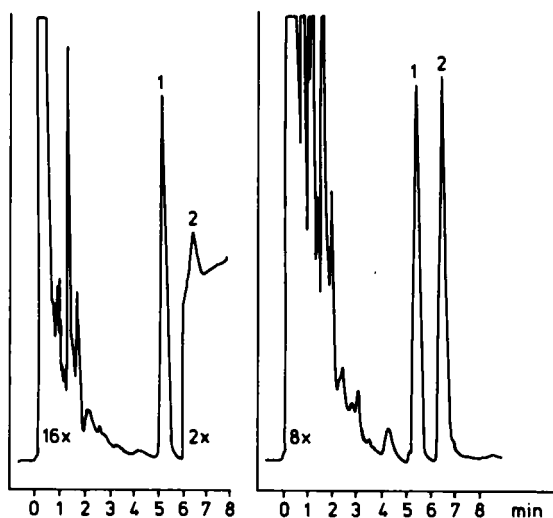


TABLE 22.2

PILOCARPINE - LIST OF ABBREVIATIONS

alk = alkaloid
 AWS = acid washed, silanized
 bi = biological fluid
 CW = Chromosorb W
 Dia = Diaport
 Epon = Epon 1001 Resin
 GP = Gas Chrom P

I.D. = inside diameter
 O.D. = outside diameter
 pil = pilocarpine
 PVP = polyvinylpyrrolidone
 qnt = quantitative
 s = separation
 S = silanized

22.2 REFERENCES

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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LIST OF ABBREVIATIONS USED FOR DESCRIPTION OF MOBILE PHASES

ACN	Acetonitrile
AcOH	Acetic acid
AmOH [*]	Amyl alcohol
BuOH [*]	Butanol
Bu ₂ O	Dibutyl ether
DEA	Diethylamine
DMFA	Dimethylformamide
EtOAc	Ethyl acetate
EtOH	Ethanol
Et ₂ O	Diethyl ether
IsoprOH	Isopropanol
(Isopr) ₂ O	Diisopropyl ether
Me ₂ CO	Acetone
MeEtCO	Methyl ethyl ketone
MeOH	Methanol
PrOH	Propanol
THF	Tetrahydrofuran
TrEA	Triethylamine

* In the Tables the prefix n, sec or t is added if it was mentioned in the original literature.

I. GENERAL PART

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Chapter 1

GENERAL ASPECTS OF HPLC OF ALKALOIDS

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For a successful analysis of alkaloids by means of HPLC a series of factors has to be considered. They are - besides those of an instrumental nature, which are beyond the scope of this book - the stationary phase, the mobile phase, the method of detection and the sample preparation. They will be discussed briefly in this chapter.

So far HPLC analysis of alkaloids has been performed by means of ion-exchange, reversed-phase, ion-pair and straight-phase chromatography. Also, some general aspects of the alkaloid analysis by means of these types of chromatography will be dealt with in this chapter.

1.1. ION-EXCHANGE HPLC

Although the ionic properties of alkaloids would make them suitable objects for ion-exchange chromatography, this technique has only found limited applications in the analysis of alkaloids.

The stationary phases employed in the HPLC are usually chemically bonded ion-exchange groups (alkylsulfonic groups) on silica gel. The analysis of some opium alkaloids on such a stationary phase has been studied by Knox and Jurand^{1,2} (see Chapter 7). Retention of the alkaloids could be altered by changes in the mobile phase, i.e. the ionic strength, the nature of the counter ion, the pH and the addition of an organic solvent to the mobile phase.

Twitchett et al.³ evaluated ion-exchange chromatography for the analysis of a wide variety of drugs (see Chapter 7). They found that retention is not only due to ion-exchange mechanisms, but partition chromatographic mechanisms also played a role. Whereas Knox and Jurand^{1,2} only used low percentages of organic solvents in the mobile phase, Twitchett et al. reported that at least 40% acetonitrile or methanol should be used to obtain good efficiency. McMurtrey et al.⁴ reported the analysis of some isoquinoline alkaloids by means of ion-exchange chromatography (see Chapter 6). They also used low percentages of organic modifiers in the mobile phase, the nature of this modifier had only little influence on the selectivity of the separation. Walton and Murgia^{5,6,7} reported the separation of various alkaloids on cation-exchange resins loaded with metal ions capable of forming complexes with ammonia (Cu^{++} , Ni^{++} , Zn^{++} , Ag^{+}). This type of chromatographic technique has not found any further applications in the analysis of alkaloids. In the appendix the properties of some commercially available ion-exchange stationary phases are summarized.

1.2. REVERSED-PHASE HPLC

Reversed-phase chromatography has most widely been used in HPLC alkaloid analysis - particularly on microparticulate silica gel with chemically bonded alkyl groups, i.e. octyl and octadecyl groups. However, in several cases it has been found that reversed-phase materials were unsuitable for the analysis of basic compounds^{8,9}. So Baker et al.¹⁰ reported highly variable column efficiency (plate number varying from 100 to 2500, see Tables 2.2 and 2.3) for various basic compounds, when using an octadecyl column. The tailing and variable plate numbers are probably caused by free silanol groups in the stationary phase.

The number of silanol groups in silica gel is about 6 per nm². For hydrocarbons directly bonded to silica, an average of about 4 silanol groups is bonded. For the smallest group - trimethylsilyl - this may even be 5, whereas for the bulky octadecyl group only 2 silanol groups are bonded per nm²^{11,12}. Unger et al.¹³ came to an even lower coverage of the silanol groups (see Table 1.1). The remaining silanol groups are shielded by the hydrocarbon group. According to Karch et al.¹⁴ the optimum coverage and shielding of the silanol groups is obtained with C₄-alkyl chains. The number of free silanol groups in long chain alkyl bonded packing material can be reduced by endcapping, i.e. the bonded phase is further treated with a small monofunctional silane, such as trimethylsilane¹⁵.

The best results in the analysis of alkaloids will generally be obtained with a stationary phase with the highest possible coverage of the silanol groups. The amount of adsorption power (free silanol groups) left in a reversed-phase material, can be tested by using the column in the adsorption mode with a non-polar mobile phase, such as heptane, and a solute such as methanol or acetone. If no adsorption is present, a symmetrical peak with a k' (capacity factor) equal to 0 is expected¹³.

The mobile phases employed in reversed-phase chromatography usually consist of water to which an organic solvent - miscible with water (mostly methanol or acetonitrile) - is added. Generally an increased polarity of the mobile phase gives a decreased solvent strength, i.e. an increased k'. For alkaloids - analyzed by means of water - methanol mixtures - systematic studies have been made¹⁶.

Karch et al.¹⁴ presented an eluotropic series for some common solvents used in reversed phase HPLC (see Table 1.2). The series is based on the retention of the solvents mentioned on an alkyl bonded stationary phase using water as eluent. The higher the value for the sol-

TABLE 1.1

SURFACE CONCENTRATION OF SOME CHEMICALLY BONDED ALKYL GROUPS IN REVERSED-PHASE SILICA GEL PACKINGS¹³.

Bonded organic functional group	Surface concentration ($\mu\text{mole}/\text{m}^2$)
Trimethylsilyl	4.5
Dimethylphenylsilyl	2.6
Triphenylsilyl	1.5
n-Butyldimethylsilyl	3.6
n-Butyldiphenylsilyl	1.8
n-Octyldimethylsilyl	3.8
n-Hexadecyldimethylsilyl	3.4
Silica gel - silanol groups	8.0

TABLE 1.2

RELATIVE RETENTIONS, (RELATIVE TO METHANOL) ON REVERSED PHASES WITH WATER AS ELUENT (ELUOTROPIC SERIES)¹⁴

Compound	Stationary phase		Compound	Stationary phase	
	C ₈	C ₁₈		C ₈	C ₁₈
Methanol	1.0	1.0	Dimethylformamide	9.4	7.6
Acetic acid	2.7	-	Acetone	9.3	8.8
Ethanol	3.2	3.1	<i>n</i> -Propanol	10.8	10.1
Acetonitrile	3.3	3.1	Dioxane	13.5	11.7
Isopropanol	8.4	8.3			

vent, the more it will reduce the retention of a sample - if the solvent is used in a mixture with water as eluent.

Bakalyar et al.¹⁷ found that the selectivity of eluents containing mixtures of water with methanol, acetonitrile or tetrahydrofuran varied for different functional groups. The selectivity could be varied by using ternary solvents. The selectivity of a reversed-phase separation on alkyl bonded phases could best be varied by altering the mobile phase; the nature of the alkyl group had only limited influence on the selectivity.

In order to reduce tailing on reversed-phase materials, basic mobile phases can be used. However, the stability of the chemically bonded groups above pH 8.5 is limited. The stability of some reversed-phase materials for various amines was studied by Wehrli et al.¹⁸. When using inorganic bases, the columns had a lifetime of only a few days. Silica gel dissolved much faster than the reversed-phase materials. Using primary, secondary and tertiary amines or ammonia in the mobile phase, a negligible decomposition of the reversed-phase materials was found. Sodium hydroxide and quaternary amines dissolved the stationary phases, particularly the silica gel, leaving the hydrocarbon groups on the remnants of the support. The attack of the silica gel lattice decreased in the series primary, secondary, tertiary amine and in the series methyl, ethyl, propyl. The water soluble triethylamine was found to be most suited as basic modifier for reversed-phase chromatography.

For chemically bonded stationary phases, column life can be improved by using a pre-injection silica gel guard column¹⁹ (see below).

To overcome the problem of tailing in reversed-phase HPLC, salts can also be added to the mobile phase - so-called ion-suppression. Ammonium carbonate, sodium acetate and sodium phosphate have been used for this purpose in the analysis of alkaloids. Also, ion-pairing has proved to be successful in alkaloid analysis. It allows analysis of alkaloids under acidic conditions, thus avoiding the problem of chemisorption of the basic compounds on the acidic silanol groups.

Addition of long alkyl chain amines in low concentrations to the mobile phase has been described as a method to improve peak performance in the analysis of basic compounds²⁰⁻²⁷. Low concentrations of tetramethyl ammonium in the mobile phase have also been reported to improve peakshape in reversed-phase HPLC^{28,29}. The beneficial effect of the addition of amines to the mobile phase was explained by the masking of free silanol groups by the amines. Several authors compared various amines for effect on the column performance^{23,25,26,27}. Gill et al.²⁷ found that an increase in chain length of the amine additive results in a significant improvement of peakshapes of basic compounds. The introduction of hydroxyl

groups in the amine considerably reduced the beneficial effect of the amine additive. Also, the geometry of the amine was an important factor. Of the isomers, triethylamine and hexylamine, the latter gave better results than the former, indicating that the primary amine interacts more strongly with the active silanol groups than does the former. Introduction of one or two further methyl groups on the nitrogen in hexylamine did not affect the interaction of the amine with the silanol groups. Bij et al.²⁵ found that the long chain amines even at very low concentrations (1 mM) were much more efficient in masking silanol groups than, for example, the more bulky triethylamine. The authors described a method to measure the silanol masking effect and found hexadecyltrimethyl ammonium bromide to be the most active compound.

Comparison of various alkyl chain lengths of the chemically bonded groups has led to the conclusion that selectivity is not changed, but that k' increases with increased alkyl chain length^{12,13,14}.

Goldberg³⁰ compared several types of octadecyl columns for their retention of neutral, acidic and basic compounds. Vastly differing chromatographic properties were found. A more extended study of the diversity of octadecyl bonded phases was made by Engelhardt et al.³¹. The suitability of the various materials for the analysis of basic compounds is also taken into consideration. An important point made by these authors is the pH of the silica gel in aqueous suspension. This pH may range from 3.8 - 9.9³². Reversed-phase materials made from such basic silica gels showed better peakshapes for basic compounds than did material prepared from a weakly acidic silica gel.

Besides stationary phases containing chemically bonded alkyl chains, such phases with cyano-alkyl and alkyl-amino chains have been used in the analysis of alkaloids. However, so far, no extensive comparative studies have been performed on the usefulness of the various reversed-phase materials for the analysis of basic compounds.

Several studies on the mechanism of retention in reversed-phase chromatography have been carried out and different theories have been developed to explain the retention mechanism (ref.33-37). Reviews on the various aspects of reversed-phase HPLC have been given by a number of authors^{12,15,36,38,39,40,41}.

An advantage of reversed-phase chromatography in the analysis of alkaloids in biological fluids is that an analysis can be carried out directly without any laborious sample clean-up procedure. However, the use of a precolumn to avoid a too rapid deterioration of the HPLC column is advisable (see Chapter 11). When using aqueous salt solutions in reversed-phase chromatography, one has to be aware of the risk of corrosion of stainless steel columns (see Table 1.3)³⁸.

TABLE 1.3

TYPICAL FOR STAINLESS STEEL CORROSIVE SOLUTIONS³⁸.

Will corrode at all concentrations	Will corrode at 10% in water
Aluminium fluoride	Aluminium chloride, aluminium nitrate
Ammonium chloride, ammonium fluoride	Ammonium diphosphate
Amyl chloride, benzoyl chloride	Ammonium perchlorate
Aqua regia (HCl + HNO ₃)	Boric acid
Chloroacetic acid	Potassium chlorate
HF, HCl, HBr and Br ₂	Sodium bicarbonate, sodium carbonate
Pb, Li, Mg halides	
KCl, KBr, NaCl, NaBr	

SCHEME 1.1

GENERAL OUTLINES OF A REVERSED-PHASE HPLC SYSTEM FOR THE SEPARATION OF BASIC COMPOUNDS

Stationary phase	Mobile phase
Octadecyl (octyl) bonded phase with low percentage of free silanol groups	ION-SUPPRESSION MODE : methanol (acetonitrile) - water containing ca. 0.01 - 0.1 M phosphate buffer, ammonium carbonate or sodium acetate (pH 4 - 7). ION-PAIR MODE : methanol (acetonitrile) - water containing ca. 0.005 M alkylsulfonate and 1% acid (acetic acid), pH 2 - 4.

Besides chemically bonded phases on silica gel supports microparticulate macroporous polymer resins also have been used in the analysis of alkaloids (see Chapters 7 and 8). A disadvantage of macroporous polymer resins is that they are not as rigid as the reversed-phase materials based on silica gel. In addition they may shrink or swell slightly - depending on the composition of the mobile phase. On the other hand they are more stable than chemically bonded phases on silica gel. They can be used in the entire pH range - and a column life of more than two years without loss in efficiency has been reported^{42,43,44}. Robinson et al.⁴⁴ presented an eluotropic series of solvents for such resins.

A summary of the properties of some of the commonly used reversed phase stationary phases is given in the appendix. The general outlines for a reversed-phase HPLC-system for basic compounds is given in Scheme 1.1.

1.3. ION-PAIR HPLC

The ionic properties of alkaloids under acidic conditions make them suitable for ion-pair chromatography. In ion-pair chromatography the alkaloidal cation is basically combined with an anion to yield an ion-pair, which can act as a neutral molecule. The ion-pair is partitioned between a mobile phase and a stationary phase during the chromatographic process. Ion-pair chromatography can be practised in the normal or in the reversed-phase mode.

The theory of the retention mechanisms in reversed-phase ion-pair chromatography has been the subject of several controversial publications. Models have been described in which the ion-pair itself is adsorbed on the stationary phase or in which the pairing ion is adsorbed on the stationary phase, thus acting as a sort of ion-exchange stationary phase⁴⁶⁻⁵⁰. Terms such as soap chromatography^{46,47} and dynamic ion-exchange chromatography⁴⁹ have been used in this connection. A more general theory for ion-pair chromatography has been given by Bidlingmeyer et al.^{51,52} and Knox and Hartwick⁵³. It explains retention by adsorption of the pairing ions (hetaerons) on the stationary phase, resulting in a charged surface. The higher the concentration of the hetaeron on the surface, the stronger the retention of opposite charged ions. Knox and Hartwick⁵³ found a linear relation between surface concentration of the hetaerons and k' of opposite charged solute ions. Solute ions with the same charge as the hetaeron showed decreasing k' upon increasing hetaeron surface concentration, eventually leading to negative k' -values. By comparison of hetaerons of different chain lengths it was observed that the k' did not depend on chain length - when the surface concentrations of the hetaerons were compared.

From loading a reversed-phase stationary phase with the hetaerons it was noticed that the

longer chain ions had larger breakthrough volumes, thus requiring longer equilibration times⁵³. In order to reduce the equilibration time, high concentrations of hetaeron could be used to load the column; subsequently the concentration of the hetaeron was reduced to the desired level, until equilibration occurred. The strongly adsorbed hetaerons were difficult to wash out of the column, e.g. dodecyl-sulfate could not be removed completely from an octadecyl stationary phase⁵³.

Straight-phase ion-pair HPLC has been applied for the analysis of alkylamines (see Chapter 15) using a stationary phase loaded with an aqueous picrate or naphthalenesulfonate solution. These counter-ions also improve the detection limit of poorly UV-absorbing compounds. Also inorganic counter-ions, such as bromide and perchlorate, have been used in straight phase ion-pair chromatography (see Chapter 15).

Reversed-phase ion-pair chromatography has also been used for the analysis of alkyl amines (see Chapter 15). Most commonly alkylsulfonates and alkylsulfates have been used as counter-ions. The influence of the alkyl-chain length of the counter-ion on the retention has been investigated⁵³⁻⁵⁸. For equal concentrations of it in the mobile phase the retention generally increases with increased alkyl-chain length of the hetaeron (see Chapter 2 and 7).

Addition of amines to the mobile phase seems to improve peakshape and to regulate the retention^{20,21,24,29,30}. A comparison of some reversed-phase stationary phases used in ion-pair HPLC has been performed^{54,56,57} (see Chapter 2 and 7).

Lurie^{56,57,58} found that the retention of closely related compounds could be best improved by changing the water content of the mobile phase or the alkyl-chain length of the pairing ion. Bidlingmeyer^{51,52} optimized a separation by using mixed pairing-ions. Reviews on ion-pair chromatography have been given^{52,59,60}.

The general outlines of a reversed-phase ion-pair HPLC-system are summarized in Scheme 1.1.

1.4. STRAIGHT-PHASE HPLC

Due to the extensive experience gained in analysis of alkaloids by TLC on silica gel plates and the initial poor peak performance of basic compounds in reversed-phase HPLC, the number of applications of silica gel as stationary phase in the HPLC of alkaloids is relatively high, as compared with other groups of compounds.

Also, because of the weakly acidic silanol groups present in silica gel, basic modifiers, such as ammonia, diethylamine and triethylamine, have often been added to the solvent systems used in TLC-analysis of alkaloids to avoid tailing caused by chemisorption (see Fig. 8.9). However, under basic conditions the stability of silica gel is poor¹⁸. Depending on the nature of the organic solvents used in combination with the basic modifier, the silica gel is dissolved at various rates. This limits the applicability of silica gel as stationary phase in the HPLC analysis of alkaloids. For certain solvent systems the column life can be extremely short - even some days.

Atwood et al.¹⁹ studied the dissolution of silica gel in the mobile phase by measuring the concentration of silica gel in the column effluent by atomic absorption. For uncoated silica gel and water as mobile phase at a flow rate of 1 ml/min the concentration of silica gel in the column effluent was 38 µg/ml. For silica gel with chemically bonded octadecyl groups, no measurable amounts of silica gel were found in the effluent under the same experimental conditions (detection limit 0.3 µg/ml). The same was true for uncoated silica

gel when methanol was used as mobile phase. However, by using a pre-injection guard column - which saturates the mobile phase with silica gel - a column could be used with acetonitrile - water (2:3) containing ammonia (pH 10.7) as mobile phase without the rapid loss of efficiency that was observed for an analytical column without a guard column. The amount of silica gel in the column effluent, when using a guard column, was about 100 µg/ml. Also, reversed-phase materials could be protected in the same way against dissolution.

Engelhardt and Müller³² reported on the differences in the physical properties, such as specific surface area, specific pore volume and average pore diameter - and on the different amounts of stationary and mobile phase per unit column volume for various commercially available silica gels. If the retention for various solutes were normalized for these factors, distinct selectivities were still noticed. This could be explained by differences in the surface pH of the silicas. Irregular ones were usually neutral or weakly acidic, whereas the spherical ones were either acidic (pH ca.4) or basic (pH ca.9) (see Table 1.4). To obtain the required and optimum selectivity, the pH of silica gel can easily be adjusted. For basic compounds more symmetrical peak shapes were obtained on silica with a basic character.

The activity of the silica gel is in HPLC of alkaloids of limited value, since the mobile phases mostly used contain a relatively high percentage of polar modifiers, such as alcohols and/or bases. Deactivation of the silica gel by water present in the samples or in the mobile phase will be swamped by the effect of the modifiers mentioned. In straight-phase chromatography a change of the mobile phase will require longer equilibration time than in reversed-phase chromatography with chemically bonded phases. A more detailed discussion on the properties of silica gel is given in the literature⁶¹⁻⁶⁴.

Aluminium oxide, which frequently has been used in TLC analysis of alkaloids, has only found limited application in HPLC.

Concerning the mobile phases used in straight-phase HPLC, the reader is also referred to the discussion on mobile phases in Volume 23A of this series: Chromatography of Alkaloids Part A: Thin-layer chromatography.

The general outlines of a straight-phase HPLC-system for basic compounds is given in Scheme 1.2.

TABLE 1.4

pH VALUES OF DIFFERENT SILICA GELS MEASURED IN A 1%(w/w) AQUEOUS SUSPENSION³².

Silica gel	pH	Regular(R) or irregular(I)	Silica gel	pH	Regular(R) or irregular(I)
Zorbax BPSil	3.9	R	Lichrosorb Si60	7.8	I
Lichrospher Si100	5.3	R	Polygosil 60-5	8.0	I
Nucleosil 100-7	5.7	R	Spherosil XOA 400	8.1	R
H 80-10 (home-made)	6.5	I	Hypersil	9.0	R
Lichrosorb Si100	7.0	I	Lichrospher Si1000	9.2	R
Porasil	7.2	I	Spherisorb S10W	9.5	R
Partisil 10	7.5	I	Lichrospher Si500	9.9	R

SCHEME 1.2

GENERAL OUTLINES OF A STRAIGHT-PHASE HPLC SYSTEM FOR THE SEPARATION OF BASIC COMPOUNDS

Stationary phase	Mobile phase
Silica gel	Dichloromethane, Chloroform, Ether (diethyl-,isopropyl-), - Tetrahydrofuran or Ethyl acetate
	Methanol or Isopropanol
	Ammonia, Diethylamine or Triethylamine (ca. 1% of the mobile phase)

1.5. DETECTION METHODS

In the HPLC analysis of alkaloids, UV detection has mostly been used. Detectors with fixed wavelength (254 and/or 280 nm) have been widely employed. These detectors are fairly sensitive - allowing detection in the ng range of alkaloids. Multiwavelength UV detectors can be used to achieve a more selective and/or more sensitive detection. However, detection in the shorter wavelength region of UV limits the number of solvents that can be used in the mobile phase. Of the polar solvents, alcohols can be used down to about 205 nm and acetonitrile and water down to a little below 200 nm. Of the non-polar solvents, alkanes can be used to about 200 nm, while moderately polar ethers have a UV cut-off point at about 220 nm.

Due to the great differences in the extinction coefficients of alkaloids - for example $E_{1\text{cm}}^{1\%} = 6$ for atropine and $E_{1\text{cm}}^{1\%} = 888$ for serpentine at 254 nm - equal amounts of alkaloids may give quite different peak areas. A chromatogram recorded by means of an UV detector may therefore be misleading in a direct comparison of the amounts of different alkaloids present based on peak areas (see also Table 7.7).

The difference in UV absorption of a compound, measured at two different wavelengths by means of a dual wavelength detector, has been used for identification purposes in HPLC of alkaloids too. The absorption ratio of a compound measured at two different wavelengths is characteristic for a compound. Baker et al.¹⁰ (ratio 254:280, Table 2.2 and 2.3) and Lurie et al.⁶⁵ (ratio 220:254, Table 7.6) applied this method to the analysis of a series of drugs.

The absorption ratio mentioned can also be used to control the purity of a compound eluted from a HPLC column and recorded as one peak. If a peak represents more than one compound, the absorption ratio will differ from that of a pure compound⁶⁶.

Fluorescence detection is highly specific and often extremely sensitive. However, its use is limited to fluorescent alkaloids - eventually to alkaloids made fluorescent by derivatization. Various chemical reactions can be used for this purpose, such as oxidation to give fluorescent oxidation products, coupling with fluorescent groups, i.e. dansyl groups, and ion-pairing with fluorescent ions, i.e. picrate, β -naphthalenesulfonate, 9,10-dimethoxyanthracenesulfonate. Pre- and post-column derivatization techniques have been described (see Chapter 4). Reviews on pre- and post-column reactions have been given⁶⁷⁻⁷².

A highly specific detection method was introduced by Westwood et al.⁷³. Using a CD (circular dichroism) spectrophotometer as detector, CD active compounds could be detected selectively. This was demonstrated for some Amaryllidaceae alkaloids (see Chapter 6).

Also, electrochemical detection has been applied in a few cases to alkaloids (see Chapters 7, 8 and 11). An advantage of the method is that it enables a selective attenuation of interfering compounds. The sensitivity is at least comparable with UV detection⁷⁴.

The most selective method of detection is probably the coupling of HPLC and MS. Eckers et al.⁷⁵ used it in connection with the analysis of alkaloids. A detailed discussion of the various detection methods in HPLC is given by Scott⁷⁶.

1.6. SAMPLE PREPARATION

Sample preparation is an important step in HPLC. It influences not only the sensitivity and the selectivity of the analysis, but also the column life and the analysis time. The general problems in alkaloid sample preparation have been dealt with in Volume 23A of this series. Some special problems connected with the analysis of alkaloids in body fluids have also been discussed there (Chapter 12), while other special problems have been dealt with in connection with the analysis of xanthine derivatives in biological fluids (Chapter 11 of this volume).

It is obvious that the cleaner the sample, the longer the column life. On the other hand extensive clean-up procedures are time consuming. A compromise can be the use of precolumns to protect the analytical column against contamination caused by impurities present in the sample. Precolumns can be prepared from less expensive material than the analytical columns, i.e. pellicular packings or large diameter particles, which can be easily dry-packed. However, microparticulate stationary phases have also been used. As HPLC analysis of alkaloids often deals with the determination of low concentrations of alkaloids in a complex matrix, some factors concerning the sample preparation which influence the detection limit are worth consideration.

Karger et al.⁷⁷ pointed out that one of the most important ways to improve the sensitivity is to inject large sample volumes. When the alkaloids to be analyzed are dissolved in the same solvent as is used as the mobile phase, as much as several hundreds of μl can be injected without loss of column efficiency. When a solvent is used from which the solute is strongly retained on the analytical column, a concentration of the alkaloids to be analyzed can be achieved on the top of the column - avoiding laborious extraction techniques. Afterwards elution can take place with a suitable mobile phase. Such a procedure can be used to enrich trace components in urine samples for instance, and it has been demonstrated for the analysis of dihydroergocristine⁷⁸. In this study it was found that for equal amounts of alkaloid sample, an increase of the volume injected from 50 μl to 7 ml only slightly increased the peakwidth. Even as large injection volumes as 165 ml gave reasonable reproducible separations, allowing analysis in the lower ppb range. By using a specially devised step-gradient elution system, a further improvement of the method was possible, allowing injection volumes as large as one liter⁷⁹. A selective detection method can reduce the interference of other compounds. On column "preconcentration techniques" have extensively been applied in several studies^{80,81}, and their fundamentals have been discussed theoretically⁸².

Van Vliet et al.⁸³ performed an enrichment of trace components by pumping the sample solution through a precolumn via a valve to the drain: the precolumn was then - via the same valve - connected to the analytical column and a stepwise gradient elution was applied. The precolumn was designed to allow rapid sampling from large volumes and to cause only negligible band spreading. For the components tested (phthalates) the best results were obtained by means of a 2 x 4.6 mm ID precolumn packed with 5 μm particles of an octadecyl bonded stationary phase. Trace enrichment from samples up to 1 l were possible. Band broadening observed for the described system was not more than can be observed for conventional 10 - 20 μl injections.

Even at the high flow rates used for pumping the sample solution through the precolumn, a complete recovery of the samples was found.

Reviews of various preconcentration methods have been given by Kirkland⁸⁴ and Frei⁸⁵. Kirkland et al.⁸⁶ investigated peak broadening caused by the injection mode, column design, detectors and guard columns.

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Chapter 2

HPLC ANALYSIS OF VARIOUS ALKALOIDS

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A number of papers have been published on the HPLC analysis of alkaloids in order to study the possibilities for such analyses in general. The papers comprise a wide variety of alkaloids and HPLC systems for their separation. The data published in these papers may be useful in connection with many separation and other HPLC problems in the analysis of alkaloids. Therefore some of the data of general interest are summarized in the present chapter. However, in other chapters of this book, HPLC systems that may be useful for alkaloid analysis in general, have also been dealt with (see Table 2.1).

2.1. ION-EXCHANGE HPLC

Walton and co-workers^{2,3,6} reported the analysis of various alkaloids by means of ligand-exchange chromatography. As stationary phases, ion-exchange materials loaded with metal-ions that are capable of giving ammonia complexes (Cu^{++} , Ni^{++} , Zn^{++} and Ag^+) were used.

A cation-exchange stationary phase for the separation of various drugs, including several alkaloids, has been tested^{7,8}. The method was found to be suitable for basic compounds. An aqueous ammonium phosphate buffer containing at least 40% methanol, or better, acetonitrile, should be used as mobile phase. Variation in the solvent ratio, ionic strength and pH could be used to vary the retention of the alkaloids.

2.2. REVERSED-PHASE HPLC

Twitchett and Moffat⁴ tested a microparticulate octadecyl stationary phase for the analysis of some drugs, including several alkaloids. For the systems tested (methanol - aqueous ammonium phosphate buffers, pH varying from 3 to 9) severe tailing was observed for basic drugs, making these systems less suitable for the analysis of such compounds.

The analysis of a series of basic drugs by means of reversed-phase HPLC was reported in connection with the identification of these compounds by dual-wavelength detection¹² (see Table 2.2). The influence of the methanol - water ratio on the retention of some alkaloids on reversed-phase packings has been studied¹⁴.

Aramaki et al.¹⁵ studied the chromatographic behaviour of some alkaloids on a macroporous styrene-divinylbenzene copolymer (see Fig.8.6 and Table 8.4). The influence of changes in the mobile phase on the retention was studied.

TABLE 2.1

ANALYSIS OF VARIOUS ALKALOIDS, DEALT WITH IN OTHER CHAPTERS.

Ref.	Ref. in other chapter	Chapter	Fig.	Table	Ref.	Ref. in other chapter	Chapter	Fig.	Table
4	21	7			15	43	8	8.6	8.4
5	22	7	7.16	7.8, 7.9	17	91	7		7.11
7	27	7			18	98	7		
8	30	7			19	99	7		
9	38	7	7.11	7.3	20	100	7		
12	56	7			22	121	7		7.6
13	60	7	7.14						

2.3. ION-PAIR HPLC

Lurie^{9,18,19,20} reported the analysis of a series of drugs of forensic interest by means of reversed-phase ion-pair HPLC (Chapter 7, Fig.7.11 and Table 7.3). Also Achari and Jacob¹⁶ studied this method in more detail. Different reversed-phase packings were tested and the influence of the mobile phase composition on the retention was studied. It was concluded that alkyl bonded phases produced longer retention than did phenyl or cyclohexyl bonded phases. The higher the organic content of the stationary phase the longer the retention. Retention is also increased by increasing the water content of the mobile phase and by increased chain length of the alkylsulfonate counter-ion.

2.4. STRAIGHT-PHASE HPLC

Verpoorte and Baerheim Svendsen¹ reported the analysis of a series of alkaloids on micro-particulate silica gel using diethyl ether or chloroform with various percentages of methanol as mobile phase (Table 2.4). The optimum wavelength of detection for fixed wavelength detectors of 254 and 280 nm for the alkaloids investigated were also reported.

Jane⁵ found highly polar mobile phases, consisting of methanol - aqueous ammonium nitrate solution - ammonia in combination with silica gel columns suitable for the analysis of drugs with a wide range of polarity (Chapter 7, Fig. 7.16 and Table 7.8 and 7.9). A similar system was employed by Baker et al.¹² (Table 2.3).

Pharmaceutical preparations containing alkaloids have been analyzed on silica gel with the mobile phase dichloromethane - methanol - ammonia¹⁰ (Table 2.5). Diethyl ether saturated with water to which a small amount of diethylamine was added has also been used for the same purpose¹³ (Table 2.6). In connection with the testing of a new detector, a separation of some alkaloids on silica gel has been reported¹¹.

Flanagan et al.²¹ reported the analysis of basic drugs on silica gel using non-aqueous ionic eluents: methanol - diethyl ether mixtures containing 0.02 - 0.1% perchloric acid or perchlorate salts. Retention and selectivity in such systems could be adjusted by the pH, ionic strength and solvent ratio. Also, bonded phases could be used in combination with such solvent systems. Basic compounds were retained only when ionized, the retention of such compounds could therefore be predicted by their pKa values. However, at low pH, retention was decreased again; the best results are therefore obtained at intermediate pH-values. Quaternary ammonium compounds gave tailing in this type of solvent system. The authors suggested that retention is caused by ionized silanol groups (ion-exchange mechanisms) but also that ion-pair formation could play a role.

TABLE 2.2 IDENTIFICATION OF SOME DRUGS BY HPLC WITH DUAL WAVELENGTH UV-DETECTION¹²
 Column, μ Bondapak C18(300x3.9 mm ID), mobile phase 0.025 M sodium dihydrogen phosphate in methanol - water (2:3), flow rate 2 ml/min.

Drug	relative retention time	absorption ratio A_{254}/A_{280}	A_{254}^c	N^d	Drug	relative retention time	absorption ratio A_{254}/A_{280}	A_{254}^c	N
Barbituric acid	0.21 ^a	19.1	2.9	-	Codeine	0.90	2.47	-	940
Sulfanilamide	0.23	5.25	2.7	4520	Bromural	0.95	14.4	0.0059	710
Phenylephrine	0.27	1.73	0.049	2680	Naloxone	0.96	2.21	0.0077	600
Theobromine	0.28	2.25	0.23	3190	Phenacetin ^b	1.00	8.42	0.49	2620
Paracetamol	0.30	8.23	1.8	3750	Heroin	1.11	1.75	-	1190
Acetylsalicylic acid	0.31	0.32	0.029	3750	Lobeline	1.11	8.52	0.0028	1920
Hydroxyamphetamine	0.32	0.94	0.043	3750	Mephesisin	1.11	3.23	0.0092	880
Phenylpropanolamine	0.34	16.0	0.0088	1420	Naphazoline	1.11	1.14	0.019	500
Theophylline	0.34	3.24	0.46	4660	Fluorescein	1.15	2.12	0.21	2220
Dimenhydrinate	0.38	3.67	0.16	5320	Methylphenidate	1.46	17.0	0.0018	320
Oxymorphone	0.41	2.80	0.046	7580	Nylidrine	1.59	1.76	0.0025	480
Ephedrine	0.41	17.1	0.0083	1150	Dihydrocodeinone	1.66	1.94	0.0029	400
Mescaline	0.43	5.41	-	1600	Ethylmorphine	1.69	2.18	0.0057	660
Caffeine	0.48	2.15	1.3	2330	Levorphanol	1.78	0.35	0.0006	370
Ectylurea	0.50	17.9	0.013	260	Chlordiazepoxide	2.02	3.69	0.045	1180
Procaine	0.51	3.0	0.61	1250	Pentazocine	2.03	0.46	0.0009	360
Amphetamine	0.52	20.4	0.019	630	Diphenylhydantoin	2.07	16.4	0.0091	630
Salicylamide	0.53	0.83	0.094	2680	Glutethimide	2.12	12.0	0.0039	1350
Nikethamide	0.58	16.5	0.15	1480	Phencyclidine	2.16	19.3	-	250
Phenacemide	0.59	33.5	0.0055	110	Levallorphan	2.35	0.44	0.0007	650
Oxycodone	0.60	2.1	0.0026	230	Phenaglycodol	2.90	16.5	0.0026	860
Morphine	0.62	1.67	0.011	950	Doxylamine	2.95	11.4	0.0093	340
Dichlorophenazone	0.66	6.0	0.026	240	Flurazepam	3.21	3.76	0.016	690
Mephenoxalone	0.67	1.79	0.0035	240	Thiopental	3.55	0.33	0.013	3150
Methocarbamol	0.72	1.43	0.0035	190	Oxymethazoline	3.62	1.00	0.0014	650
Dimethyltryptamine	0.75	1.89	-	750	Methaqualone	3.94	2.51	0.049	920
Methylamphetamine	0.75	6.50	0.0021	230	Phenazocine	4.05	0.72	0.0011	780
Tetrahydrozoline	0.77	9.46	0.0054	360	Oxazepam	4.05	6.16	0.061	1510
Phenmetrazine	0.80	13.3	0.0021	670	Thiamylal	4.58	0.31	0.016	2370
Dihydrocodeine	0.81	1.08	0.0043	270	Methohexital	4.80	9.0	0.0015	2600
Dihydromorphinone	0.84	2.0	0.0070	460	Papaverine	7.06	3.0	0.029	2440
Nicotine	0.85	14.4	0.056	990	Diazepam	9.56	6.04	0.10	2650
Mephentermine	0.88	7.67	0.002	450					

^a The column void volume was slightly less than 0.21

^b Retention time 6.8 min

^c Absorbance of a 10- μ l injection of a 1.0 mg/ml solution of the drug

^d Number of theoretical plates

TABLE 2.3

IDENTIFICATION OF SOME DRUGS BY HPLC WITH DUAL WAVELENGTH UV-DETECTION¹²

Column μ Porasil (300x3.9 mm ID), mobile phase methanol - 2 M ammonia - 1M ammonium nitrate (27:2:1), flow rate 2.0 ml/min. A_{254}/A_{280} = absorption ratio UV.

Drug	relative retention time	absorption ratio		
		A_{254}/A_{280}	A_{254}^c	N^d
Noscapine	0.53 ^a	0.61	0.035	-
Phenacetin	0.53	0.84	0.051	-
Naloxone	0.56	0.82	0.025	-
Papaverine	0.56	1.06	0.070	-
Benzphetamine	0.58	2.10	0.069	-
Piminodine	0.58	3.02	0.16	-
Cocaine	0.61	0.86	-	-
Phenazocine	0.61	0.24	0.0081	-
Procaine	0.61	0.44	0.048	-
Nylidrin	0.61	0.75	0.013	-
Levallorphan	0.64	0.12	0.0026	-
Methylphenidate	0.67	9.50	0.0062	-
Pentazocine	0.67	0.16	0.0049	-
Phendimetrazine	0.67	8.00	0.010	-
Ethinamate	0.70	1.00	0.0020	-
Phenmetrazine	0.72	31.0	0.040	-
Meperidine	0.75	30.7	0.0030	710
Quinine	0.75	0.62	0.0042	1,700
Promethazine	0.76	2.21	0.14	710
Diphenhydramine	0.77	90.0	0.059	1,600
Methapyrilene	0.77	1.89	0.13	750
Phenylpropanolamine	0.78	65.0	0.0021	-
Heroin	0.80	0.64	-	1,800
Methadone	0.83	1.57	0.072	1,140
Phencyclidine	0.83	22.0	-	1,900
Thioridazine	0.83	2.08	0.20	840
Amphetamine	0.86	60.0	0.0039	940
Oxymorphone	0.86	1.17	0.0044	110
Doxylamine	0.89	17.7	0.0017	2,330
Ethylmorphine	0.92	1.15	0.020	2,440
Hydroxyamphetamine	0.92	0.38	0.011	1,080
Propylhexedrine	0.92	4.0	0.0005	290
Oxycodone	0.92	1.13	0.0029	100
Codeine	1.00	0.88	-	450
Morphine	1.00 ^b	1.09	0.016	470
Dimethyltryptamine	1.09	0.77	-	3,190
Methamphetamine	1.19	31.0	0.0034	1,862
Ephedrine	1.20	52.0	0.0034	1,050
Phenylephrine	1.22	0.50	0.0045	466
Dihydrocodeinone	1.28	0.93	0.0085	1,170
Ethioheptazine	1.31	27.4	0.0022	1,510
Mescaline	1.31	2.93	-	2,140
Xylometazoline	1.33	8.67	0.0034	2,290
Mephenteramine	1.36	36.3	0.0038	1,370
Dihydrocodeine	1.36	0.53	0.0065	1,290
Oxymetazoline	1.36	0.31	0.012	1,370
Tetrahydrozoline	1.42	16.1	0.038	1,460
Dihydromorphinone	1.43	1.09	0.0091	970
Strychnine	1.54	3.22	0.0045	370
Dextromethorphan	1.56	0.14	0.0022	1,750
Naphazoline	1.61	0.49	0.062	1,210
Levorphanol	1.64	0.12	0.0013	1,210

^a The column void volume was slightly less than 0.53

^b Morphine was used as standard, retention time 3.5 min

^c Absorbance of a 10- μ l injection of a 1.0 mg/ml solution

^d Number of theoretical plates

TABLE 2.4

RETENTION TIMES OF SOME ALKALOIDS¹Column, Merckosorb SI 60, 5 μ m (300x2 mm ID), detection UV 254 nm.

RT = retention time; FR = flow-rate.

Alkaloid	Solvent system											
	Chloroform-methanol											
	9 : 1		8 : 2		7 : 3		8 : 2		7 : 3		6 : 4	
	RT	FR	RT	FR	RT	FR	RT	FR	RT	FR	RT	FR
Quinine	-	-	6.4	0.81	5.9	0.75	3.9	0.85	2.7	1.43	2.2	1.34
Quinidine	-	-	6.5	0.81	6.2	0.75	3.7	0.85	2.9	1.43	2.4	1.34
Cinchonine	-	-	10.6	0.81	11.0	0.75	5.9	0.85	5.1	1.43	4.2	1.34
Cinchonidine	-	-	9.0	0.81	9.0	0.75	4.5	0.85	3.7	1.43	3.0	1.34
Atropine	-	-	-	-	-	-	-	-	9.4	1.38	7.4	1.20
Scopolamine	2.0	0.81	1.5	0.81	1.5	0.75	1.7	1.50	1.2	1.38	1.3	1.20
Cocaine	3.4	0.81	2.1	0.81	3.9	0.75	3.2	1.50	2.0	1.38	2.9	1.20
Strychnine	6.9	0.81	3.9	0.81	4.7	0.75	-	-	9.4	1.43	7.3	1.34
Brucine	6.8	0.81	3.9	0.81	4.6	0.75	-	-	17.2	1.43	13.1	1.34
Morphine	8.6	0.81	3.5	0.81	3.3	0.75	4.0	1.50	4.3	1.43	4.3	1.34
Codeine	4.6	0.81	2.7	0.81	2.9	0.75	3.4	1.50	3.6	1.43	3.4	1.34
Thebaine	4.1	0.81	2.7	0.81	3.0	0.75	2.7	1.50	3.7	1.43	3.7	1.34
Heroin	2.3	0.81	1.7	0.81	1.7	0.75	2.1	1.50	2.2	1.43	2.2	1.34
Narceine	-	-	7.5	0.81	5.7	0.75	-	-	-	-	8.5	1.34
Noscapine	1.1	0.81	1.0	0.81	1.1	0.75	0.6	1.50	0.6	1.43	0.7	1.34
Papaverine	1.1	0.81	1.1	0.81	1.2	0.75	0.8	1.50	0.8	1.43	0.8	1.34
Emetine	1.1	0.81	1.1	0.81	1.2	0.75	0.6	1.50	0.7	1.43	0.8	1.34
Cephaeline	-	-	5.9	0.81	8.6	0.75	-	-	-	-	8.1	1.34
Serpentine	-	-	-	-	-	-	-	-	-	-	-	-
Alstonine	-	-	-	-	-	-	-	-	-	-	-	-
Reserpine	2.2	0.38	1.0	0.81	1.1	0.75	2.9	0.33	3.0	0.33	2.2	0.40
Yohimbine	3.7	0.38	1.5	0.81	1.7	0.75	4.6	0.33	3.8	0.33	2.8	0.40
Raubasine	2.2	0.38	1.0	0.81	1.1	0.75	2.9	0.33	2.9	0.33	2.3	0.40
Tetrahydroalstonine	2.1	0.38	1.0	0.81	1.1	0.75	2.5	0.33	2.7	0.33	2.2	0.40

TABLE 2.5

RETENTION DATA OF VARIOUS DRUGS¹⁰Column, Partisil, 10 μ m (250x4.6 mm ID), mobile phase methanol - dichloromethane (3:1) containing 1% 29% ammonia, flow rate 0.7 ml/min, detection UV 254 nm.

	k'	Tailing Factor (%)		k'	Tailing Factor (%)
Theophylline	0.08*	50.0	Brompheniramine	1.92	50.0
Sodium Sulfacetamide	0.16	25.0	Chlorpheniramine	1.92	55.6
6-Hydroxydopamine	0.54	-	Hydroquinidine	2.50	50.0
Ethaverine	0.92	25.0	Amphetamine	2.66	58.8
Papaverine	0.92	25.0	Phenylephrine	2.66	35.7
Tropicamide	0.92	66.7	Ephedrine	3.25	19.2
Caffeine	0.92	25.0	Strychnine	3.33	25.0
Theobromine	0.96	29.4	Dextromethorphan	3.58	-
Scopolamine	1.00	47.0	Antazoline	4.00	25.0
Pyrilamine	1.33	58.8	Atropine	4.13	18.8
Epinephrine	1.50	60.0	Homatropine	4.42	20.9
Phenylpropanolamine	1.66	64.0	Naphazoline	9.58	17.9
Quinidine	1.71	50.0	Xylometazoline	13.58	24.0
Quinine	1.71	55.6	Oxymetazoline	15.25	53.3
Codeine	1.88	40.0			

* retention time 2.59 min.

TABLE 2.6

SEPARATION OF SOME ALKALOIDS AND DRUGS (SEE ALSO FIG.7.14)¹³Column, Partisil 5 μ m (250x4.6 mm ID), mobile phase diethyl ether 95% saturated with water + 0.4% diethylamine, flow rate 2 ml/min, detection UV 254 nm.

Alkaloid	k'
Aconitine	1.51
N-methylephedrine	0.58
Ephedrine	2.89
Emetine	2.36
Cephaeline	3.95
Ethylmorphine	4.29
Codeine	5.58
Papaverine	2.15
Noscapine	0.35
Narceine	0.58
Scopolamine	4.11
Atropine	8.93
Homatropine	9.55
Quinine	4.34
Strychnine	6.32
Caffeine	5.62
Phenytaine	6.73
Sulfanilamide	8.82
Phenobarbital	14.9

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II. SPECIAL PART

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CHAPTER 3

PYRROLIDINE, PYRROLIZIDINE, PYRIDINE, PIPERIDINE AND QUINOLIZIDINE ALKALOIDS

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Nicotine is an alkaloid which is commonly found in urine and, therefore, nicotine has been included in several studies concerning the analysis of drugs and drugs of abuse in urine samples (Table 3.1). Analysis of tobacco alkaloids in general has been performed by HPLC; also in investigations on pyrrolizidine alkaloids, many of which possess hepatotoxic, carcinogenic, teratogenic and mutagenic properties, has HPLC been successfully applied.

3.1. REVERSED-PHASE HPLC

Pietrzyk et al.⁹ studied the effect of solute ionization on the chromatographic retention. As stationary phase the polystyrene-divinylbenzene copolymer XAD₂ was used. One of the model compounds was nicotine, for which the retention at various pH of the mobile phase was determined.

Hanks et al.²² analyzed nicotine-containing pesticides by means of an octadecyl column with methanol - 0.05 M ammonium hydrogen phosphate (pH 7.5) (3:2) as mobile phase.

Quantitative analysis of the alkaloids in tobacco was reported by Piade and Hoffmann²³. Two octadecyl columns were coupled in series and the alkaloids separated with a gradient of an increasing amount of acetonitrile in a triethylamine - phosphoric acid buffer (Fig.3.1). The influence of the pH on the separation of the alkaloids was studied; optimum results were obtained at pH 7.56. The results were compared with a GLC and a spectrophotometric method.

Analysis of the major tobacco alkaloids has been performed on an octadecyl column using a triethylamine - phosphoric acid buffer (pH 7.25) to which 40% methanol was added (Fig.3.2) (24). The method was compared with a GLC method. Several extraction solvents were compared as to their effectiveness. An 0.025 M phosphate buffer (pH 7.8) was found to be suitable.

In a series of papers, Segall and co-workers described various HPLC methods for the analysis of pyrrolizidine alkaloids in plant material. A cyano-type column in combination with tetrahydrofuran - 0.01 M ammonium carbonate was used (Fig.3.3) for the isolation and identification of some *Senecio* alkaloids^{11,12,13,20}. A gradient of 13% tetrahydrofuran, increasing to 26%, gave good separations. Similar results could be obtained with an isocratic system containing 16% tetrahydrofuran.

An octadecyl column was also employed and methanol - potassium dihydrogen phosphate buffer (pH 6.3) as mobile phase. The advantage of this system over the above mentioned one was that it allowed detection at a shorter wavelength (218 nm), i.e. at the absorption maxima of the alkaloids (Fig.3.4)¹⁶. It could also be applied to preparative¹⁷ and semipreparative²¹ separations. Huizing and Malingre¹⁵ purified and separated pyrrolizidine alkaloids on a polystyrene-divinylbenzene resin (XAD₂) using acidic methanol - water mixtures. An increased percent-

tage of methanol suppressed the tailing of the peaks.

An analytical separation of pyrrolizidine alkaloids on a styrene-divinylbenzene resin has been reported by Ramsdell and Buhler²⁵. Such a stationary phase can be used at relatively high pH (Fig.3.5).

The analysis of pyrrolizidine alkaloids in *Radix symphyti* was reported by Tittel et al. (18,26). Analysis of the N-oxides could be performed on an octadecyl column (Fig.3.6). The reduced alkaloids could be separated on an alkylamino column. Because of interfering peaks, the latter method was not suitable for the analysis of plant material. The N-oxides could also be separated on an alkylamino stationary phase using acetonitrile - water (98:2) as mobile phase (Fig.3.7)²⁶.

3.2. STRAIGHT-PHASE HPLC

Watson⁸ analyzed nicotine and cotinine extracted from urine on a silica gel column using ethyl acetate - isopropanol - ammonia (80:3:0.4) as mobile phase. Maskarinec et al.¹⁰ separated the same alkaloids - after isolation from biological fluids by adsorption on a XAD₂ resin - on a silica gel column with dioxane - isopropanol - ammonia (80:3:0.4).

3.3. DETECTION

For the sensitive detection of pyrrolizidine alkaloids it is necessary to use solvent systems that enable detection at the absorption maxima of these alkaloids, below 220 nm^{16,18}. Baker et al.¹⁴ reported the identification of various drugs by means of their relative retention times in combination with the absorbance ratio, calculated from the peak heights observed with 254 and 280 nm UV-detection.

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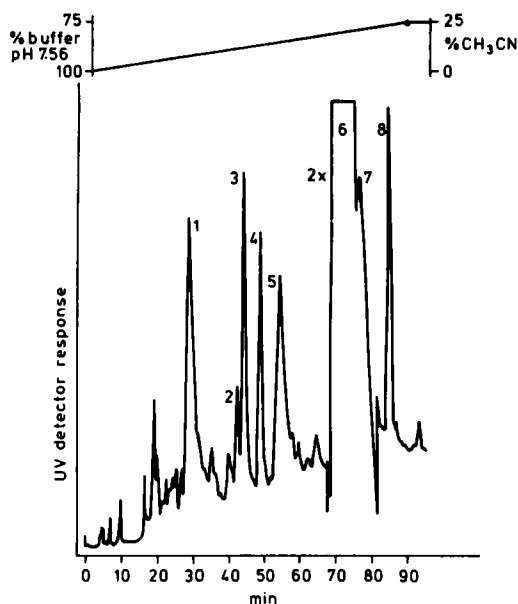


Fig. 3.1. HPLC separation of Tobacco alkaloids²³
Pre-column Bondapak C18, followed by two columns Lichrosorb RP18 (250x2 mm ID) in series, mobile phase linear gradient of 0-25% acetonitrile (90 min) in 0.07 M triethylamine (pH 7.56 with phosphoric acid), flow rate 1.5 ml/min, detection UV 254 nm. Peaks: 1, nornicotine; 2, anabasine; 3, cotinine; 4, N'-formyl-nornicotine; 5, anatabine; 6, nicotine; 7, myosmine; 8, 2,3'-dipyridyl. (Reproduced with permission from ref.23, by courtesy of Marcel Dekker, Inc.)

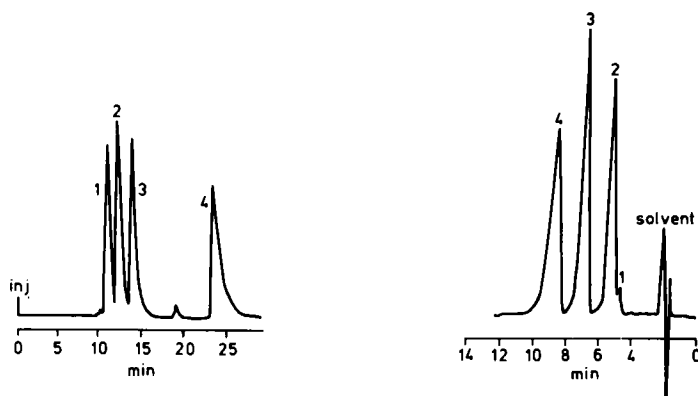


Fig. 3.2. HPLC separation of major Tobacco alkaloids²⁴
Column μ Bondapak C18 (300x4 mm ID), mobile phase methanol - water (2:3) buffered with 0.2% phosphoric acid to which triethylamine is added until pH 7.25, flow rate 0.5 ml/min, detection UV 254 nm. Peaks: 1, nornicotine; 2, anabasine; 3, anatabine; 4, nicotine.

Fig. 3.3. HPLC separation of some *Senecio* alkaloids¹¹
Column μ Bondapak CN (300x4 mm ID), mobile phase tetrahydrofuran - 0.001 M ammonium carbonate (pH 7.8)(16:84), flow rate 1.8 ml/min, detection UV 235 nm. Peaks: 1, unknown; 2, retrorsine; 3, seniciphylline; 4, senecionine.

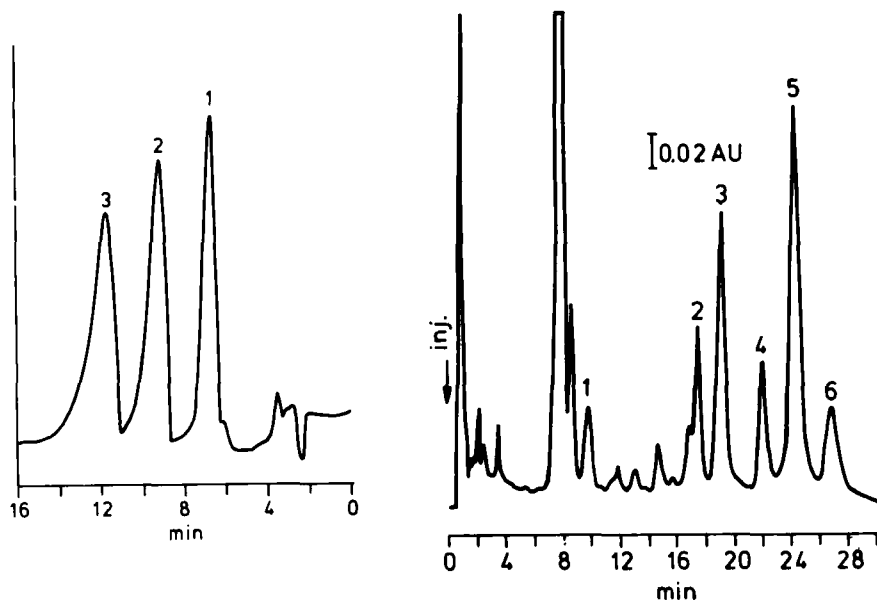


Fig. 3.4. HPLC separation of some *Senecio* alkaloids¹⁶
Column μ Bondapak C18 (300x4 mm ID), mobile phase methanol - potassium dihydrogen phosphate (pH 6.3)(55:45), flow rate 1.2 ml/min, detection UV 225 nm. Peaks: 1, retrorsine; 2, seneciphylline; 3, senecionine. (Reproduced with permission from ref. 16, by courtesy of Marcel Dekker, Inc.)

Fig. 3.5. HPLC separation of *Senecio jacobaea* alkaloids²⁵
Column PRP-1 (styrene-divinylbenzene resin)(150x4.1 mm ID), mobile phase acetonitrile - 0.1 M ammonia, 20 min. linear gradient from (1:9) to (3:7), flow rate 1 ml/min, detection UV 220 nm. Peaks: 1, jacoline; 2, jacozone; 3, jacobine; 4, jaconine; 5, seneciphylline; 6, senecionine.

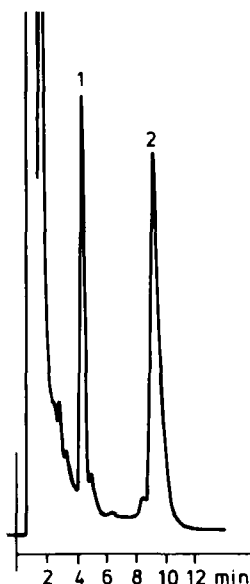


Fig. 3.6. HPLC separation of some *Symphytum* alkaloids¹⁸
Column MN-Nucleosil C18 10 μ m (300x4 mm ID), mobile phase methanol - water (45:55), flow rate 2 ml/min, detection UV 220 nm. Peaks: 1, echimidine N-oxide; 2, symphytine N-oxide. (Reproduced with permission from ref. 18, by courtesy of Hippokrates Verlag)

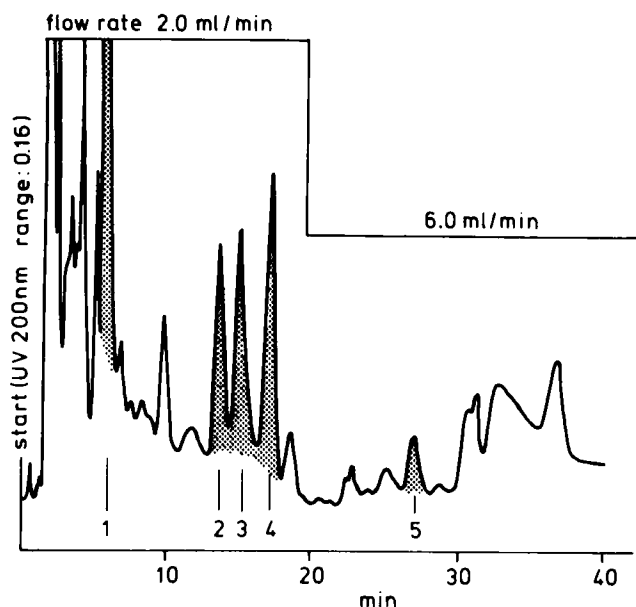


Fig. 3.7. HPLC separation of some *Symphytum* alkaloids²⁶
 Column μ Bondapak NH₂ (300x4 mm ID), mobile phase acetonitrile - water (92:8), flow rate gradient from 2 ml/min to 6 ml/min in 18 min, detection UV 200 nm. Peaks: 1, allantoin; 2, symphytine N-oxide; 3, echimidine N-oxide; 4, acetyl-lycopsamine N-oxide; 5, lycopsamine N-oxide. (Reproduced with permission from ref. 23, by courtesy of Hippokrates Verlag)

TABLE 3.1

PYRROLIDINE, PYRROLIZIDINE, PYRIDINE, PIPERIDINE AND QUINOLIZIDINE ALKALOIDS IN THE CONTEXT OF HPLC ANALYSIS OF DRUGS OF ABUSE (CHAPTER 7)

Alkaloids	Ref.	Ref. in Chapter 7
Nicotine	3	21
Nicotine	4	22
Nicotine	6	30
Nicotine	7	32
Nicotine, lobeline	14	56

TABLE 3.2

HPLC ANALYSIS OF VARIOUS COMPOUNDS INCLUDING PYRROLIDINE, PYRROLIZIDINE, PYRIDINE, PIPERIDINE AND QUINOLIZIDINE ALKALOIDS

Alkaloid	Aims	Stationary phase	Column dim. LxID mm	Mobile phase	Ref.
Nicotine,quinine, strychnine,opium and tropane alkaloids	Separation on ionexchange resins (ligand-exchange LC)	hydrolyzed Poragel PT, loaded with Cu^{++}	470x6.3	0.06M NH_4OH in 33% EtOH	1,2,5
		Bio-Rad, PC20, loaded with Cu^{++}	470x6.3	0.2M NH_4OH in 33% EtOH	
				0.05M NH_4OH in 33% EtOH	
				0.03M NH_4OH in 33% EtOH	

TABLE 3.3

HPLC ANALYSIS TOBACCO ALKALOIDS

Alkaloid	Aims	Stationary phase	Column dim. LxID mm	Mobile phase	Ref.
Nicotine,cotinine	Analysis in urine	Micropak SI 10	250x2	EtOAc-isoprOH- NH_4OH (80:3:0.4)	8
Nicotine	Effect solute ionization on retention	XAD ₂ , 45-65 μm	250x2	0.01M phosphate buffers of various pH, ionic strength made up to 0.1M with NaCl in ACN- H_2O (1:9)	9
Nicotine,nornicotine, cotinine	Analysis in biological fluids	Zorbax-Sil	250x4.6	Dioxane-isoprOH- NH_4OH (80:3:0.4)	10
Nicotine	Analysis in pesticides	μ Bondapak C18 or Lichrosorb RP18, 10 μm	300x4 250x4.1	MeOH-0.05M $(\text{NH}_4)_2\text{PO}_4$ (pH 7.5)(3:2)	22
Nicotine,nornicotine, anabasine,anatabine, cotinine,N'-formyl- nornicotine,myosmine, 2,3'-dipyridyl	Analysis in tobacco (Fig.3.1)	Lichrosorb RP18	250x2 (two in series)	A 0.07M TrEA (pH 7.56 with H_3PO_4) B ACN linear gradient 0-25% B in A in 90 min	23
Nicotine,nornicotine, anabasine,anatabine	Analysis in tobacco and fresh plant material (Fig.3.1)	μ Bondapak C18	300x4	0.2% H_3PO_4 (pH 7.25 with TrEA) in $\text{MeOH-H}_2\text{O}$ (2:3)	24

TABLE 3.4

HPLC ANALYSIS PYRROLIDINE, PYRROLIZIDINE, PYRIDINE, PIPERIDINE AND QUINOLIZIDINE ALKALOIDS

Alkaloid	Aims	Stationary phase	Column dim. LxID mm	Mobile phase	Ref.
Retrorsine, seneciphylline, senecionine, ridelline, jacobine, jacoline, jaconine	Analysis alkaloids in <i>Senecio</i> species	μ Bondapak CN	300x4	THF - 0.01M $(\text{NH}_4)_2\text{CO}_3$ (pH 7.8) (16:84) or linear gradient 13-26% THF in 30 min	11,12 13,20
Echimidine, echinatine, symphytine	Purification and separation alkaloids from Boraginaceae	XAD ₂ 50-100 μm or 74 μ 250 μm	260x13, 610x20, 150x10	MeOH - H ₂ O in various ratios, gradient ⁵ and pH MeOH - H ₂ O (1:1) + HCl (pH 3.5)	15
Retrorsine, ridelline, senecionine, seneciphylline, senkerkine	Identification in <i>Senecio</i> species (Fig.3.4)	μ Bondapak C18	300x3.9	MeOH-0.01M KH_2PO_4 (pH 6.3) (1:1), (55:45), (3:2)	16
Retrorsine, senecionine, seneciphylline	Preparative LC of <i>Senecio</i> alkaloids	500/C18 column	2 columns	MeOH-0.005M KH_2PO_4 (pH 6.3) (3:2)	17
Echimidine, symphytine and their N-oxides	Analysis in <i>Symphytum</i> plant material (Fig.3.6)	MN Nucleosil NH ₂ 10 μm MN Nucleosil C18 10 μm	300x4 300x4	CH ₂ Cl ₂ sat. with 1% aq. $(\text{NH}_4)_2\text{CO}_3$ -isobutanol (20:1) MeOH-H ₂ O (45:55)	18
Matrine, oxymatrine, sophoranol, sophocarpine N-oxide, amagryne	Analysis in <i>Sophorae radix</i>	Lichrosorb NH ₂	no details	ACN-aq. H_3PO_4 (pH 2.0)	19
Echiumine, intermediine, jacobine, jacoline, jaconine, jacozone, lycopsamine, senecionine, seneciphylline, sincamidine	Isolation by semipreparative HPLC from plant material	μ Bondapak C18	300x7.8	MeOH-0.01M KH_2PO_4 (7:33)	21
Jacobine, jacoline, jaconine, retrorsine, senecionine, seneciphylline	Separation on styrene-divinylbenzene resin (Fig.3.5)	PRP-1	150x4.1	ACN-0.1M NH_4OH (1:3) or linear gradient (1:9) to (3:7)	25
Echimidine-, lycopsamine-, acetyllycopsamine-, symphytine-N-oxide	Analysis in <i>Symphytum</i> plant material (Fig.3.7)	μ Bondapak NH ₂	300x4	ACN-H ₂ O (92:8)	26

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Chapter 4

TROPANE ALKALOIDS

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4.1. TROPINE ALKALOIDS*

Most of the work performed so far on tropine alkaloids concerns the analysis of such alkaloids in pharmaceutical preparations (Table 4.5). Special methods have been investigated in order to lower the detection limit of such alkaloids^{13,16,28,33,35,37,41,43,45}. Reviews on the application of derivatization techniques in LC given by Frei and Santi¹⁶ and Frei³⁹, include some examples of tropine alkaloids. The analysis of atropine and its degradation products has also been investigated^{25,30,32,38}.

4.1.1. ION-EXCHANGE HPLC

Ligand-exchange chromatography of alkaloids by means of ion-exchange materials loaded with metal ions was described by Walton and Murgia^{6,10,17}. The technique is discussed in Chapter 7. Instead of a HPLC system using a diol column^{35,41}, Huen and Thevenin⁴⁵ found a microparticulate sulfonic acid cation-exchange material to be better for a selective separation of tropane alkaloids prior to post-column fluorimetric ion-pair derivatization (Fig.4.1). Atropine and scopolamine could be separated from its major decomposition products: apoatropine, tropic acid, tropine and scopoline.

4.1.2. REVERSED-PHASE HPLC

Honigberg et al.¹⁵ described the HPLC analysis of antispasmodic mixtures, containing i.a. atropine and scopolamine. The retention behaviour was studied by changing three operating parameters, i.e. the stationary phase, the methanol - water ratio, and the pH of the mobile phase. A pellicular octadecyl and a phenyl column were used. It was found that more basic mobile phases - containing ammonium carbonate - led to tailing and multiple peaks for atropine and scopolamine. Generally better results were obtained on a phenyl column.

Lund and Hansen³² studied the separation of atropine and some of its decomposition products using microparticulate medium polarity reversed-phase materials as stationary phase, i.e. stationary phases containing chemically bonded cyano or alkylamino groups. Atropine and apoatro-

* Because atropine and l-hyoscyamine behave similar in all HPLC systems, the term atropine will be used in the text and in all tables and figures to describe both alkaloids.

pine could be separated on a cyano column, whereas tropic acid and atropic acid were separated on an alkylamino column. Coupling of a cyano and an alkylamino column in series enabled the separation of all test compounds (Table 4.1). A mixed bed column (cyano : alkylamino 2:1) was found to allow fast separation; however, atropine could not be determined quantitatively because it was eluted close to the solvent front. The problem could be solved by increasing the length of the cyano column to 15 cm. Column switching was used to improve the detection limit of apoatropine and still allow a quantitative determination of atropine (Fig.4.2).

Gfeller et al.^{35,41} used a microparticulate chemically bonded diol stationary phase in combination with an exclusively aqueous mobile phase. It allowed post-column ion-pair derivatization of the separated alkaloids. By changing the pH and the ionic strength of the buffer, the capacity factors of the alkaloids could be varied.

Atropine was included in a series of alkaloids which were separated on a macroporous styrene-divinylbenzene copolymer⁴⁷ (Chapter B, Table 8.4).

4.1.3. ION-PAIR HPLC

For most of the HPLC analyses of tropine alkaloids, ion-pair chromatography has been applied. Burdo²² analyzed methylscopolamine using sodium decylsulfate as ion-pairing reagent. Walters²⁶ used a similar method for the determination of atropine and scopolamine in tablets. Octanesulfonic acid (0.01 M) served as the pairing-ion in an acetate buffer of pH 3.5 containing 34% acetonitrile. The analysis was performed on an octadecyl column. For qualitative work a decrease of the percentage of acetonitrile to 28 was preferred (Table 4.2). Brown et al.^{27,30} used heptanesulfonic acid (0.01 M) as pairing-ion and an aqueous mobile phase of pH 3.40 containing 35% acetonitrile in the analysis of anticholinergic drugs²⁷, and atropine³⁰ on microparticulate octadecyl columns.

Hartmann³⁸ continued the work started by Burdo²², mentioned above, and used sodium decylsulfate and dioctylsulfosuccinate as ion-pairing reagents in the analysis of methylscopolamine in neomycine containing veterinary preparations (Fig.4.3).

Achari and Jacob⁵⁰ studied several parameters that have an influence on the retention of basic drugs in ion-pair HPLC, i.e. atropine and scopolamine. Some general conclusions were drawn concerning the nature of the pairing-ion, the type of chemically bonded stationary phase and the composition of the mobile phase (Chapter 2).

For the analysis of atropine and its major acidic decomposition products, Kreilgard²⁵ used 0.01 M tetrabutylammonium sulphate as pairing-ion in a mobile phase of acetonitrile - 0.05 M acetate buffer (pH 5.5)(1:4)(Table 4.3).

Santi et al.¹³ tested various stationary phases for straight-phase ion-pair partition chromatography. Picric acid served as precolumn pairing-ion for the separation of atropine, scopolamine and ergotamine because of its strong UV-absorption, which enabled a 100-300 times improvement of the detection limit of the poorly UV-absorbing tropine alkaloids (see detection). Good results were obtained with microparticulate Kieselguhr, but the reproducibility of the column performance was difficult because of variation in the quality of the Kieselguhr. Microparticulate silica gel with a pore size of 100 or 1000 Å was found to be better as support for the pairing-ion, but low flow rates had to be used (0.2 ml/min)(Fig.4.4). Silica gel with a smaller pore size gave poor results, due to the mechanical instability of the system at the rather high linear velocities used.

TABLE 4.1

RETENTION DATA AND DETECTION LIMITS FOR ATROPINE AND ITS DEGRADATION PRODUCTS³²

Compound	k'	minimum detectable amount (ng)	Compound	k'
Atropine	0.57		β -Belladonnine	5.9
Apoatropine	2.0	5	β -Isatropic acid	3.0
Tropic acid	2.4	40	Scopolamine	0.36
Atropic acid	3.0	2	Homatropine	0.44
α -Belladonnine	3.5			

Column Nucleosil 5 CN (50x4.6 mm ID) and Nucleosil 5 NH₂ (50x4.6 mm ID) connected in series, mobile phase 0.05 M sodium acetate buffer (pH 5) - methanol (3:1), detection UV 254 nm.

TABLE 4.2

SEPARATION OF SOME TROPANE ALKALOIDS²⁶

Compound	k'	Compound	k'
Tropic acid	0.65	Methylatropine	3.63
Scopolamine	2.70	Atropine	4.00
Homatropine	2.76	Hyoscyamine	4.00
Scopolamine N-oxide	2.88	Cocaine	11.6
Methylscopolamine	3.24	Benzatropine	not eluted

Column μ Bondapak C18 (300x3.9 mm ID), mobile phase 28% acetonitrile in 0.01 M aqueous octane-sulfonic acid adjusted to pH 3.5, flow rate 1 ml/min, detection UV 230 nm.

TABLE 4.3

SEPARATION OF SOME TROPANE ALKALOIDS²⁵

Compound	k'	Compound	k'
Atropine	0.2	Atropic acid	4.5
Belladonnine	0.3	β -Isatropic acid	6.2
Tropic acid	1.5	4-Methylbenzoic acid	6.5
Apoatropine	2.5	(internal standard)	

Column Lichrosorb RP8, 5 μ m (100x4.6 mm ID), mobile phase 0.01 M tetrabutylammonium sulphate in 0.05 M acetate buffer - acetonitrile (4:1) at pH 5.5, flow rate 1 ml/min, detection UV 254 nm.

Huen et al.²⁸ found the optimum pH for ion-pair separations with picric acid to be 5-6 in their investigations, when the effect of the variation of picric acid concentration and temperature was studied.

Gfeller et al.^{33,37} described automatization of precolumn derivatization for the systems mentioned above.

Post-column derivatization with ion-pairing technique has also been used to improve the detectability of tropine alkaloids (see detection). The technique was used by Lawrence et al.⁴³ after separation of the alkaloids on a microparticulate silica gel column with a mobile phase of chloroform and methanol, containing the weakly ion-pairing butyric acid.

Crommen⁴⁴ investigated the retention of organic compounds on silica gel using aqueous mobile phases. A retention model was presented, based on the distribution of ion-pairs. Applications to the separation of some tropine alkaloids are shown in Fig.4.5.

4.1.4. STRAIGHT-PHASE HPLC

The first HPLC separation of tropine alkaloids was performed on a silica gel column by means of tetrahydrofuran - 28% ammonia (100:1)¹. Verpoorte and Baerheim Svendsen¹⁹ separated some tropine alkaloids with diethyl ether - methanol - diethylamine (90:10:1) on microparticulate silica gel (Fig.4.6). Rather large amounts of alkaloids had to be injected due to the poor chromophore, which caused severe tailing on pellicular silica gel columns. Chloroform containing mobile phases did not permit useful separations of the alkaloids because of large differences in capacity factors of atropine and scopolamine. Also, by means of a mobile phase consisting of diethyl ether and diethylamine, separations were achieved⁴² (Chapter 7, Fig.7.14).

Achari and Theimer²⁴ used methanol - dichloromethane (3:1), to which 1% of 29% ammonia was added, as mobile phase and a microparticulate silica gel column. Atropine, homatropine and scopolamine showed different capacity factors.

Straight-phase separations by means of polar mobile phases were used by Jane¹² (Chapter 7, Table 7.8).

Aigner et al.¹⁸ separated some drugs, including tropane alkaloids, on silica gel columns impregnated with silver iodide. By using gradient elution, multicomponent mixtures of drugs could be separated.

4.1.5. DETECTION

A major problem in the analysis of tropine alkaloids by HPLC is their poor chromophore. The decomposition products tropine and scopoline completely lack any chromophore. UV detection at 254 nm has, according to Stutz and Sass¹ a detection limit of about 1 µg, and RI detection of about 50 µg. Walters²⁶ found a wavelength of 230 nm to be the optimum compromise between greater absorptivity of the alkaloids and increasing background absorbance of the mobile phase (detection limit 0.5 µg). Brown and Sleeman³⁰ reported a detection limit of 200 ng for atropine at 254 nm.

Because of the low absorbance of tropine alkaloids, Santi et al.^{13,28} developed an ion-pair separation using the strong UV absorbing picric acid as pairing-ion. Compared to a reversed-phase separation followed by detection at 210 nm, a fifty-fold enhancement of the detection limit was observed using the picric acid ion-pair technique and detection at 254 nm. The detection limit for atropine was 5 ng and for scopolamine 50 ng. The non UV absorbing scopoline could be detected at a level as low as 2.5 ng. By measuring at the UV maximum of picric acid - 345 nm - interference of the signals of non-pairing compounds was suppressed. Gfeller et al.^{33,37} developed the technique to an automated pre-column derivatization method.

Gfeller et al.^{35,41} also developed an automatic post-column derivatization method. A fluorescent pairing-ion - 9,10-dimethoxyanthracene-2-sulfonate (DAS) - was used to improve the detection limit of atropine. The best results were obtained with an exclusively aqueous mobile phase, to which the reagent was added as an aqueous solution. Subsequently the ion-pair was extracted with chloroform or dichloromethane. An increase in the percentage of methanol in the mobile phase reduced the sensitivity. The method was found to be 200 times more sensitive than UV detection at 208 nm; the minimum detectable amount was 200 pg. Peak broadening caused by the post-column reactor was about 40%. To meet the requirement of a selective separation with a mobile phase containing only small amounts of organic solvents, Huen and Thevenin⁴⁵ preferred ion-exchange separation (Fig.4.2).

Lawrence et al.⁴³ described a simpler post-column ion-pair derivatization technique, whereby the alkaloids were separated by means of an organic mobile phase on silica gel. The column eluate and the aqueous DAS solution were mixed and the two immiscible phases separated. About 50% of the organic phase was led to the fluorimetric detector. Various parameters influencing the bandwidth were investigated, i.a. the influence of methanol in the mobile phase. An increasing methanol content deteriorated the signal to noise ratio, and the most useful range was 0-15% methanol. The detection limit for atropine was found to be 40 ng.

Hashimoto et al.³⁶ described a capacitance-conductance detector for HPLC; it had a detection limit of 5 μ g for scopolamine.

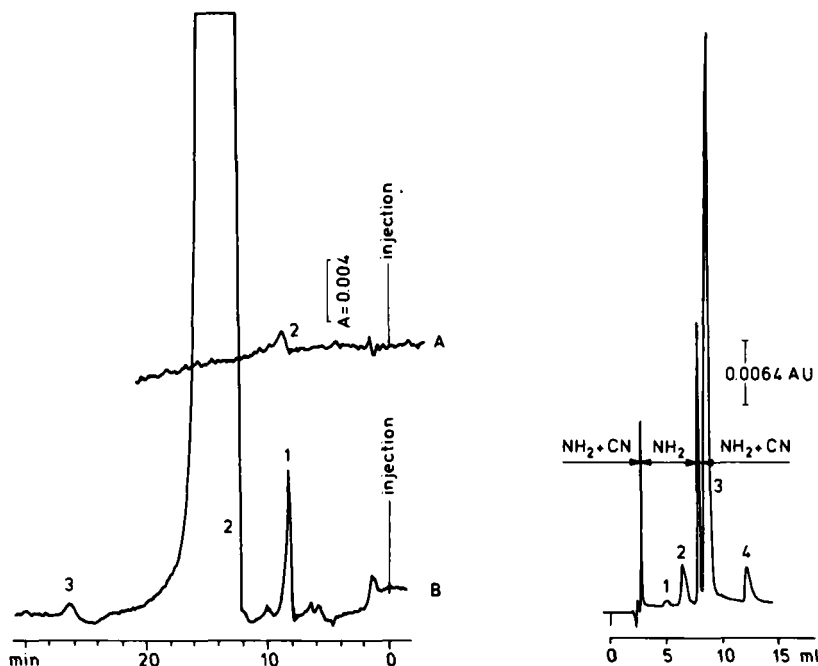


Fig. 4.1. HPLC analysis atropine with post-column fluorimetric ion-pair derivatization to improve the detection limit⁴⁵
 Column Nucleosil 10 SA (300x4 mm ID), mobile phase methanol - 0.2 M aqueous diammonium hydrogen phosphate (pH 3)(1:9), flow rate 2 ml/min, column temperature 85°C, detection UV 254 nm (1), fluorimetric detection after post-column derivatization with Na-DAS (excitation 383 nm, emission 446 nm)(2). Peaks: 1, scopolamine; 2, atropine; 3, apoatropine. (Reproduced with permission from ref. 45)

Fig. 4.2. HPLC analysis atropine and its degradation products using column switching³²
 Columns Nucleosil 5NH₂ (50x4.6 mm ID) and Nucleosil 5CN(100x4.6 mm ID), mobile phase methanol - 0.05 M sodium acetate buffer (pH 5)(1:3), detection UV 254 nm.(see also Table 4.1). Peaks: 1, tropic acid; 2, atropic acid; 3, atropine; 4, apoatropine.

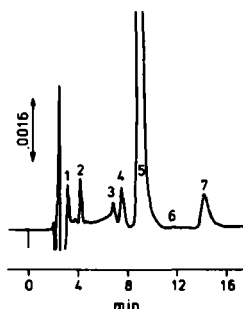


Fig. 4.3. HPLC analysis methylscopolamine and some added impurities³⁸
 Column μ Bondapak C18 (300x3.9 mm ID), mobile phase 0.01 M dioctylsulfosuccinate sodium salt and 0.01 M ammonium nitrate in 95% ethanol - water (55:45), pH adjusted to 3.5 with glacial acetic acid, flow rate 1 ml/min, detection UV 254 nm. Peaks: 1, tropic acid; 2, atropic acid; 3, DOSS artifact; 4, scopolamine; 5, methylscopolamine; 6, aposcopolamine; 7, methylaposcopolamine. (Reproduced with permission from ref. 38, by courtesy of Journal Association of official analytical chemists).

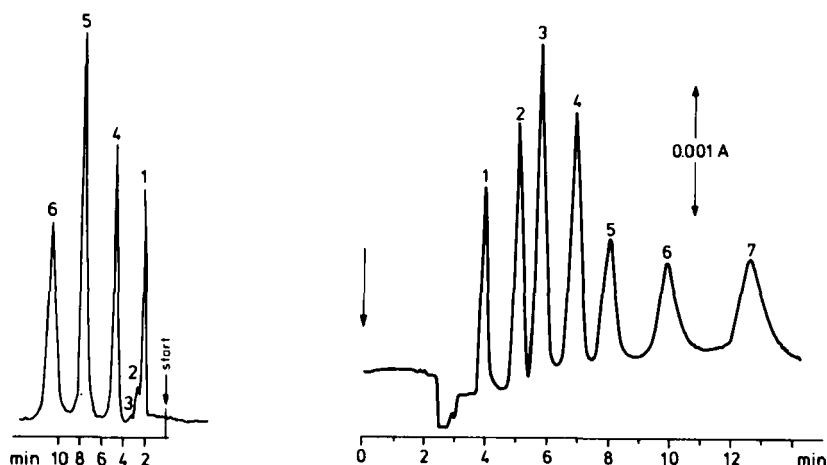


Fig. 4.4. HPLC analysis tropine alkaloids²⁸

Column Silica gel Si100 5 μ m (100x3 mm ID) impregnated with 0.06 M picric acid (pH 6), mobile phase chloroform saturated with the stationary phase 0.06 M picric acid, flow rate 0.2 ml/min, detection UV 345 nm. Peaks: 1, dodecylbenzene (t_0); 2, apoatropine; 3, ergotamine; 4, atropine; 5, ergotamine; 6, scopolamine.

Fig. 4.5. HPLC analysis anticholinergics⁴⁴

Column Lichrospher Si100 10 μ m (200x4 mm ID), mobile phase 0.1 M sodium phosphate buffer pH 2.2 containing 1.9% n-amylalcohol, linear velocity 1.6 mm/sec., detection UV 254 nm. Peaks: 1, scopolamine; 2, atropine; 3, benactyzine; 4, adiphenine; 5, methylatropine; 6, oxypropyrium; 7, oxyphenonium.

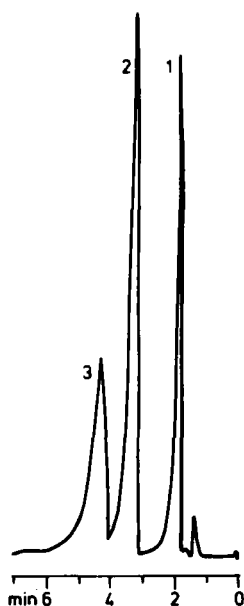


Fig. 4.6. HPLC analysis of some tropine alkaloids¹⁹

Column Partisil 5 μ m (300x4.6 mm ID), mobile phase diethyl ether - methanol - diethylamine (90:10:1), flow rate 2.29 ml/min, detection UV 254 nm. Peaks: 1, scopolamine; 2, apoatropine; 3, atropine.

TABLE 4.4

HPLC ANALYSIS OF VARIOUS COMPOUNDS INCLUDING TROPINE ALKALOIDS

Alkaloid *	Other Compounds	Aims	Stationary Phase	Column Dim. LxID mm	Mobile Phase	Ref.
A,S,22 other alkaloids		Analysis alkaloids	Merckosorb Si60, 5 μ m	300x2	CHCl ₃ -MeOH(9:1),(8:2),(7:3) Et ₂ O-MeOH(8:2),(7:3),(6:4)	5
A,opium alkaloids, quinine,cinchonine,strychnine,nicotine,coc		Separation on ion-exchange resins (ligand-exchange LC)	Hydrolyzed Poragel PT loaded with Cu ²⁺	470x6.3	0.06M NH ₄ OH in 33% EtOH	
			Bio-Rad,PC20,loaded with Cu ²⁺	470x6.3	0.2M NH ₄ OH in 33% EtOH 0.05M NH ₄ OH in 33% EtOH 0.03M NH ₄ OH in 33% EtOH	6,10, 17
A,benzotropine, various alkaloids	Various drugs of forensic interest	Analysis drugs of abuse(Table 7.8)	Partisil 6 μ m	250x4.6	MeOH-2M NH ₄ OH-1M NH ₄ NO ₃ (27:2:1) MeOH-0.2M NH ₄ NO ₃ (3:2)	12
S,Cinchona alkaloids,brucine,strychnine,emetine,reserpine,yohimbine,caffeine		Detection with conductance detector	Silica gel 10 μ m		CHCl ₃ -MeOH-hexane(7:3:10)	36
A,S,codeine, papaverine,quinine,caffeine,ephedrine	Various drugs	Retention behaviour basic drugs in ion-pair HPLC	μ Bondapak C18 μ Bondapak Phenyl μ Bondapak CH μ Bondagel Chromegabond C8 Chromegabond C ₆ H ₁₁	300x4	0.005M heptanesulfonic acid in H ₂ O-MeOH-AcOH(50:49:1) (pH 4.0)	50
A,codeine,morphine,dihydrocodeine,quinine,quinidine,caffeine,theophylline,ajmaline	Various basic drugs	Separation of basic drugs on silica gel with non-aqueous ionic eluents	Spherisorb S5W Silica	250x4.9	MeOH-hexane(85:15)containing 0.10% HClO ₄	66

* For abbreviations see footnote Table 4.5

TABLE 4.5

HPLC ANALYSIS TROPINE ALKALOIDS IN PHARMACEUTICAL PREPARATIONS

Alkaloid *	Other Compounds	Aims	Stationary Phase	Column Dim. LxID mm	Mobile Phase	Ref.
A,H,S,apoA,trop		Separation	Sil-X	1000x4.5	THF-28% NH ₄ OH(100:1)	1
HMe,codeine,dihydro-codeinone,ephedrine,strychnine	Various analgesics, antihistaminics and antitussives	Determination in cough-cold mixtures	Corasil C18	1220x2.3	ACN-1%NH ₄ OAc(3:2)(pH 7.4)	7
A,S,ergotamine		Separation with ion-pair LC	Spherosil X08,5-10 μ m loaded with 0.03M picric acid and buffer pH 5	100x2.8	CHCl ₃ sat. with 0.05M picric acid in pH 5 buffer	
			Silicagel 100,5 μ m loaded with 0.06M picric acid and buffer pH 5	100x2.8	CHCl ₃ sat. with 0.06M picric acid in pH 6 buffer	13,16
A,S	Chlordiazepoxide, propantheline, isopropamid,clidinium, phenobarbital,prochlorperazine	Determination of antispasmodic mixtures	Corasil C18 or Corasil Phenyl	1220x2.3	MeOH-1% aq. (NH ₄)H ₂ PO ₄ (6:4) (pH 5.85), (1:1) (pH 5.50), (2:3) (pH 5.50), MeOH-1% aq. (NH ₄)H ₂ PO ₄ -1% aq. (NH ₄)HPO ₄ (3:1:1) (pH 8.20), (2:1:1) (pH 7.90), (4:3:3) (pH 7.60) MeOH-0.5% aq. (NH ₄) ₂ CO ₃ (3:2) (pH 8.65), (1:1) (pH 8.70) (2:3) (pH 8.80)	15
A,S,ergotamine,ergotaminine,caffeine	Butalbital,phenobarbital	Separation on silver impregnated silica gel	Lichrosorb Si100 5 μ m, imp. with 1.09% AgI	not given	CHCl ₃ -DEA(99.99:0.01) A CHCl ₃ -hexane(1:1) B CHCl ₃ -MeOH-DEA(90:10:0.5) linear gradient 16-92% B in A (1.5-2.5 min.)	18
A,S,apoA		Separation	Partisil 5 μ m	300x4.6	Et ₂ O-MeOH-DEA(90:10:1)	19

A,H,S,quinine,quinidine, dihydroquinidine, xanthines, strychnine, ephedrine, codeine, papaverine	Various drugs	Analysis in pharmaceuticals	Partisil 10 μ m	250x4.6	CH ₂ Cl ₂ -MeOH(1:3) with 1% 29% NH ₄ OH	24
A,apoA,trop ac,atrop ac,B, β -isatropic ac		Separation atropine and degradation products (Table 4.3)	Lichrosorb RP8,5 μ m	100x4.6	0.01M tetrabutylammonium in 0.05M aq. acetate buffer-ACN (4:1) (pH 5.5)	25
A,H,S,AME,SMe,trop ac,S N-ox,coc,benztropine	Lidocaine	Determination in tablets (Table 4.2)	μ Bondapak C18	300x3.9	0.01M 1-octanesulfonic acid (pH 3.5)-ACN(66:34), (72:28)	26
A	Various drugs	Separation anticholinergic drugs	μ Bondapak C18	300x3.9	0.01M heptanesulfonic acid-ACN(65:35)	27
A,S,B,apoA,scop,ergotamine, dihydroergotamine, caffeine	Barbiturates, pizotifene	Separation with ion-pair HPLC (Fig.4.4)	Lichrosorb Si100,5 μ m loaded with 0.06M picric acid (pH=6)	150x3	CHCl ₃ sat. with 0.06M picric acid (pH 6)	28,33,37
A,trop ac		Separation	μ Bondapak C18	300x3.9	0.01M heptanesulfonic acid (pH 3.40)-ACN(65:35)	30
A,H,S, α -B, β -B,apoA,trop ac,atrop ac, β -isatropic ac		Determination atropine and its degradation products (Table 4.1, Fig.4.2)	Nucleosil 5CN and Nucleosil 5NH ₂ in series	50x4.6	MeOH-0.025M NaOAc buffer (pH 5)(1:3)	32
A,emetine,ephedrine, dihydroergotamine, bromocryptine	Various drugs	Post-column derivatization (fluorescent ion-pairs)	Lichrosorb DIOL,10 μ m Lichrosorb RP8,10 μ m	250x4 100x4.6	0.1M phosphate buffer (pH 3) MeOH-0.02M phosphate buffer (pH 3)(3:2)	35,41
SMe,S,apoS,apoSMe,trop ac,atrop ac	Neomycin, benzphetamine	Determination in veterinary formulations (Fig.4.3)	μ Bondapak C18	300x3.9	0.01M Na-decylsulfate, 0.01M NH ₄ NO ₃ in MeOH-H ₂ O(3:2) 0.01M Na-diethylsulfosuccinate, 0.01M NH ₄ NO ₃ in 95% EtOH-H ₂ O(55:45) (pH 3.5)	38
A,H,S, various alkaloids	Sulfanilamide, phenytoine, phenobarbital	Identification pharmaceuticals (Fig.7.14)	Partisil PXS 5/25	250x4.6	Et ₂ O sat. with 50-100% H ₂ O +0.05-0.8% DEA	42
A,ergotamine	Hydroxyatrazine	Post-column derivatization	Lichrosorb Si60,5 μ m	60x3	0.1M butyric acid in CHCl ₃ -MeOH(9:1)	43
A,S,AME	Various drugs	Ion-pair chromatography (Fig.4.5)	Lichrospher Si100, 5 μ m	200x4	0.1M Na-phosphate buffer (pH 2.2) +1.9% AmOH	44
A,S,apoA		Post-column derivatization (Fig.4.1)	Nucleosil 10SA	300x4	MeOH-0.2M aq. (NH ₄) ₂ HPO ₄ (1:9)	45

HMe,codeine,morphine, noscapine,papaverine, thebaine		Determination in pharmaceutical pre- parations	Nucleosil 5C8	120x4.6	ACN-0.01M phosphate buffer (pH 5.0)(2:3)	54
SMe	Pyrilamine,pheni- ramine	Analysis in tablets	Partisil 10 ODS	250x4.6	ACN-2.85mM ethylenediamine buffer(pH 7.44)(1:1)	61
A,S,theophylline	Phenobarbital	Analysis in pharma- ceutical prepara- tions	Spherisorb 5µm	250x4	MeOH-0.05M tetramethylam- monium phosphate buffer(pH 2.0) (21:10)	68,69

* Abbreviations used in Tables 4.4 and 4.5

A	atropine,hyoscyamine
AMe	methylatropine
apoA	apoatropine
atrop ac	atropic acid
B	belladonnine
coc	cocaine
H	homatropine
HMe	methylhomatropine
S	scopolamine
SMe	methylscopolamine
S N-ox	scopolamine N-oxide
apoS	aposcopolamine
scop	scopoline
trop	tropine
trop ac	tropic acid

4.2. PSEUDOTROPINE ALKALOIDS

Most of the investigations on HPLC analysis of cocaine and related compounds concern the abuse of cocaine. A series of methods has been described for the identification of street drugs - including cocaine (Table 4.11). HPLC systems used for the analysis of drugs of abuse are discussed in the Chapter 7^{4,9,11,12,20,23,30,53,58,59,60,65,66,67} (see also Tables 7.6, 7.8 and 7.11). A review on the analysis of cocaine has been given⁶⁴. Although HPLC should be a well suited method for the analysis of cocaine metabolites in biological material, only two papers seem to have been published on this matter^{31,48}. The separation of the four possible diastereoisomers of cocaine was achieved by Olieman et al.⁴⁶ and Lewin et al.⁴⁹.

4.2.1. ION-EXCHANGE HPLC

Ion-exchange chromatography has been used for the analysis of street drugs - including cocaine - in a number of investigations^{3,9,20,21}. Walton et al.^{6,10,17} applied ligand-exchange chromatography for alkaloids, i.a. for cocaine. None of the methods mentioned was designed especially for cocaine, but rather for street drugs in general. They are discussed in more detail in Chapter 7.

4.2.2. REVERSED-PHASE HPLC

Reversed-phase HPLC was used by Jatlow et al.³¹ for the analysis of cocaine and its metabolites in urine (Fig.4.7). An amount of 0.1 µg/ml cocaine could be detected by using a micro-particulate octadecyl column and an acidic mobile phase consisting of 0.25 M potassium dihydrogen phosphate (pH 2.7) containing 17% acetonitrile. For the analysis of cocaine in plasma, an octadecyl column has been employed in combination with the mobile phase methanol - 0.05 M potassium phosphate buffer (pH 6.6)(3:1), using tetracaine as internal standard⁴⁸. Noggle and Clark⁶³ reported a method for the identification of *cis*- and *trans*-cinnamoylcocaine in illicit cocaine. The alkaloids and some of their degradation products were separated on an octadecyl type of column, using methanol - aqueous phosphate buffer (pH 3)(1:2) as mobile phase.

Trinler and Reuland³⁴ used a chemically bonded diphenylsilyl stationary phase for the identification of cocaine and some local anaesthetics, commonly used as adulterants in cocaine samples. A mobile phase of acetonitrile - water (85:15) containing 1% ammonium carbonate was used. The same system was also used for semi preparative work - to allow further identification of the separated drugs by means of IR spectroscopy. To study the hydrolysis of cocaine in urine and plasma samples, Fletcher and Hancock⁵⁵ used an octadecyl column and methanol - water containing phosphoric acid to give pH 3.8 as mobile phase. Above pH 7 a significant hydrolysis of cocaine to benzoylecgonine took place. This may explain the difference which has been reported in the literature in the estimations of unchanged cocaine in body fluids.

Jane et al.⁵⁶ obtained unsatisfactory results for analysis of cocaine and related compounds when using silica gel or octadecyl modified silica gel as stationary phase. However, good separations and peak performance were obtained with a chemically bonded dimethylsilyl phase and methanol - aqueous 0.1 M ammonium nitrate (2:3)(pH 4.3) as mobile phase (Table 4.6).

TABLE 4.6

HPLC RETENTION DATA OF COCAINE AND RELATED COMPOUNDS⁵⁶
(relative retention times(RRT) were calculated with respect to cocaine(retention time 2.7 min))

Compound	RRT	Compound	RRT
Procaine	0.65	Butacaine	1.47
Chloroprocaine	0.67	<i>trans</i> -cinnamoylcocaine	1.49
Lignocaine	0.70	Amydracaine	1.69
Pyrrocaine	0.74	Phenacaine	1.72
Benzoyllecgonine	0.77	Cinchocaine	3.00
Dimethocaine	0.77	Cyclomethycaine	3.00
Octacaine	0.77		
Propoxycaine	0.80	Morphine	0.45
Prilocaine	0.83	Codeine	0.52
Mepivacaine	0.83	O-acetylmorphine	0.61
Orthocaine	0.89	Heroin	0.91
Cocaine	1.00	Acetylcodeine	0.92
Benzocaine	1.13		
Butanilicaine	1.13	Ephedrine	0.58
Piperocaine	1.17	Caffeine	0.60
<i>cis</i> -Cinnamoylcocaine	1.18	Amphetamine	0.69
Leucinecaine	1.26	Methylamphetamine	0.69
Proxymetacaine	1.32	Cyclizine	2.58
Amylocaine	1.41	Dipipanone	2.58

Column Lichrosorb RP2, 5 μ m (150x4.6 mm ID), mobile phase methanol - 0.1 M ammonium nitrate (2:3) adjusted to pH 4.3 with 2 M hydrochloric acid, flow rate 1.5 ml/min, detection UV 279 nm.

TABLE 4.7

RETENTION VOLUME OF COCAINE AND RELATED COMPOUNDS²³
(retention volume (Rr) relative to cocaine, see also Table 7.3)

Compound	Rr	Compound	Rr
Antipyrine	0.27	Cocaine	1.00(26.4 ml)
Procaine	0.34	Methaqualone	1.11
Benzocaine	0.45	Mecloqualone	1.31
Lidocaine	0.62	Tetracaine	2.60

Column μ Bondapak C18 (300x4 mm ID), mobile phase methanol - acetic acid - water (40:1:59) (pH 3.5) containing 0.005 M *n*-heptanesulfonic acid, flow rate 2 ml/min, detection UV 254 nm.

4.2.3. ION-PAIR HPLC

Lurie and co-workers^{23,58,59,60,67} used ion-pair chromatography for the analysis of street drugs. The results obtained for cocaine and some local anaesthetics on an octadecyl column using heptanesulfonic acid as pairing-ion in a mobile phase of methanol - water - acetic acid (40:1:59) are given in Table 4.7 (see also Table 7.6). Olieman et al.⁴⁶ separated the four cocaine diastereoisomers on a microparticulate octadecyl column by means of a mobile phase of tetrahydrofuran - water (1:4) containing 0.005 M *n*-heptanesulfonic acid (Fig.4.8). Lichrosorb RP18 was found to be less suitable as stationary phase than Nucleosil C18 because of tailing and peak broadening.

4.2.4. STRAIGHT-PHASE HPLC

Lewin et al.⁴⁹ separated the four isomeric cocaines by straight-phase chromatography

(Fig.4.9). HPLC was preferred over GLC because of the decomposition of some of the alkaloids during GLC. Some other straight-phase systems for drugs of abuse are dealt with in Chapter 7^{4,12}.

4.2.5. DETECTION

The UV spectrum of cocaine shows maxima at 229, 274 and 281 nm (in ethanol). The latter two maxima have only low intensity and are, therefore, not suitable for a sensitive detection. A detection at 230-235 nm has usually been preferred^{31,46,48,49}, thus limiting the choice of the mobile phase. Olieman et al.⁴⁶ found the detection limit of cocaine at 235 nm to be 2.8 ng in their system. Jatlow et al.³¹ detected cocaine both at 200 nm and at 235 nm. The former wavelength gave a 2.5 times more sensitive detection of cocaine. The debenzoylated metabolites (ecgonine, norecgonine and methylecgonine) had insufficient UV absorption for UV detection - even at 200 nm. Comparison of peak height absorbance at the wavelength of detection could be used for further verification of the identity.

Jane et al.⁵⁶ preferred UV detection at 279 nm, because it allowed detection of relatively small amounts of the strong UV absorbing cinnamoylcocaine in cocaine samples.

Baker et al.⁴⁰ used the ratio of absorbance at 254 and 280 nm to characterize drugs of forensic interest - and also cocaine. Similarly, Lurie et al.⁶⁶ used the 220/254 absorbance ratio (Table 7.6). Noggle and Clark⁶³ applied this method to the identification of cis- and trans-cinnamoylcocaine in illicit cocaine samples (254/280 ratio).

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TABLE 4.8

HPLC ANALYSIS COCAINE IN COMBINATION WITH OTHER COMPOUNDS

Alkaloid *	Aims	Stationary phase	Column Dim. LxID mm	Mobile phase	Ref.
Coc, opium alkaloids, quinine, cinchonine, caffeine	Separation by means of dynamic coating HPLC	Corasil I and II, dynamically coated with Poly G-300(2%)	1000x1	Heptane-EtOH(1:1) with different percentage saturation with Poly G-300	2
Coc, 23 other alkaloids	Analysis alkaloids	Merckosorb Si60, 5 μ m	300x2	CHCl ₃ -MeOH(9:1), (8:2), (7:3) Et ₂ O ³ -MeOH(8:2), (7:3), (6:4)	5
Coc, atropine, opium alkaloids, nicotine, strychnine, quinine, cinchonine	Separation on ion-exchange resins (ligand-exchange LC)	Hydrolyzed Poragel PT, loaded with Cu Bio-Rad PC20, loaded with Cu ⁺⁺	470x6.3 470x6.3	0.06M NH ₄ OH in 33% EtOH 0.2M NH ₄ OH in 33% EtOH 0.05M NH ₄ OH in 33% EtOH 0.03M NH ₄ OH in 33% EtOH	6, 10, 17
Coc, amyllocaine, benzocaine, butacaine	Separation basic drugs on silica gel with aqueous solvents	Lichrosorb Si100, 5 μ m	150x5	MeOH-H ₂ O(7:3) containing NH ₄ -formate, NH ₄ NO ₃ or Na-formate in various concentrations and pH	29
Coc, benzoylecgonine, morphine, heroin, OAc-morphine, benzoic acid	Stability coc and heroin in pharmaceutical dosage form	μ Bondapak C18	300x4	ACN-0.015M Na ₂ HPO ₄ (pH 3.0)(1:3)	51

TABLE 4.9

HPLC ANALYSIS OF COCAINE AND RELATED ALKALOIDS

Alkaloids *	Aims	Stationary phase	Column Dim. LxID mm	Mobile phase	Ref.
Coc, pseudococ, allococ, allpseudococ	Separation of diastereoisomers (Fig. 4.8)	Nucleosil C18, 5 μ m	150x4.0	THF-H ₂ O(1:4) containing 0.005M heptanesulfonic acid and 2% AcOH	46
Coc, pseudococ, allococ, allpseudococ	Separation of diastereoisomers (Fig. 4.9)	Partisil 10 PXS	250x4.6	Heptane-isoprOH-DEA(25:75:0.1)	49
Coc	Analysis in <i>Erythroxylum</i> plant material	Silica gel ODS C18, 8 μ m	250x4	MeOH-0.05M phosphate buffer (pH 7) (32:68)	52

* For abbreviations see footnote Table 4.11

Coc,benzoylecgonine,
ethyl-p-aminobenzoateStability coc in pharmaceuti-
cal preparations at various pH

Bondapak CN

300x4

MeOH-0.02M NH_4OAc (3:1)

62

TABLE 4.10

HPLC ANALYSIS OF COCAINE AND RELATED ALKALOIDS IN BIOLOGICAL MATERIAL

Alkaloids *	Other compounds	Aims	Stationary phase	Column Dim. LxID mm	Mobile phase	Ref.
Coc,morphine,co- deine,caffeine, theophylline		Analysis urine	BOP(no further details)		Heptane-prOH(9:1)	8
Coc,benzoylecgo- nine,norcoc,ben- zoylnorecgonine		Determination in urine (Fig.4.7)	Partisil 10 ODS	250x4.6	ACN-0.25M KH_2PO_4 (pH 2.7)(17:83)	31
Coc,benzoylecgo- nine,A,S,morphine, caffeine	Various local anaesthetics and analgesics	Analysis coc in plasma	ODS-HC SIL-X-1	250x2.6	MeOH-0.05M phosphate buffer(pH 6.6)(3:1)	48
Coc,benzoylecgo- nine		Hydrolysis coc in biolo- gical fluids	Hypersil 5 ODS,5 μm	100x4.6	MeOH- H_2O (55:45),pH 3.8 with H_3PO_4	55
Coc,papaverine, yohimbine,heroin, strychnine,caffeine	Various drugs	Analysis papaverine in blood	Micropak CN-10	300x4	Hexane- CH_2Cl_2 -ACN-propylamine (50:25:25:0.1)	57
Coc,opium alka- loids,caffeine, quinine	Various drugs	Determination heroin in blood	Lichrosorb Si60,5 μm	300x4	ACN-MeOH-(MeOH-NH ₄ OH(2:1))-(AcOH -MeOH(1:1))(75:25:0.040:0.216)	65

*For abbreviations see footnote Table 4.11

TABLE 4.11

HPLC ANALYSIS OF COCAINE IN CONNECTION WITH THE ANALYSIS OF DRUGS OF ABUSE

Alkaloids*	Other compounds	Aims	Stationary phase	Column Dim. LxID mm	Mobile phase	Ref.
Coc, various opium alkaloids, quinine, quinidine	Procaine, anileridine, methapyrilene	Analysis drugs of abuse	Zipax SAX	1000x2.1	A 0.01M boric acid buffer pH 9.5 with 1M NaOH B 0.01M KH_2PO_4 buffer pH 6.0 with 1M NaOH gradient A+B(85:15) to B, linear 5 or 10% per min	3
Coc, various opium alkaloids, quinine, LSD, mescaline	Procaine, benzocaine, various other drugs	Identification street drugs	Corasil II, 37-50 μm	500x2.3	Cyclohexane-MeOH-cyclohexylamine (98.3:1.5:0.2), (94.5:4.5:1) A Skelly B-95% EtOH-dioxane-cyclohexylamine (991.3:50:25:13) B idem (686:100:200:14) linear gradient from A to B	4
			Al_2O_3 , Woelm B18, 18-30 μm	500x2.3	Cyclohexane-cyclohexylamine (98.8:0.2)	4
Coc, various opium alkaloids, caffeine, strychnine, quinine, ephedrine	Procaine, lignocaine, barbiturate, paracetamol	Analysis illicit heroin preparations (Fig. 7.2)	Zipax SCX	1200x2.1	A 0.2M boric acid buffer, pH 9.3 with 40% NaOH B 0.2M boric acid buffer-ACN-prOH (86:12:2), pH 9.8 with 40% NaOH linear gradient 0-100% B in 6 min	9
Coc, various drugs of abuse (Table 7.8)	Local anaesthetics, various other drugs	Separation drugs of abuse	Partisil 6 μm	250x4.6	MeOH-2M NH_4OH -1M NH_4NO_3 (27:2:1) MeOH-0.2M NH_4NO_3 (3:2)	12
Coc, morphine, heroin, methadone		Screening of drugs of abuse	Bondapak C18/Corasil	610x2	ACN-H ₂ O (9:1), (65:35), (1:1) all containing 0.1% $(\text{NH}_4)_2\text{CO}_3$	14
Coc, morphine, nicotine, ephedrine, caffeine, quinine, tubocurarine	Various drugs	Evaluation ion-exchange and reversed-phase columns for the analysis of drugs	Partisil SCX 10 m	250x4.6	0.5, 0.1, 0.05, 0.01M $(\text{NH}_4)\text{H}_2\text{PO}_4$ buffers of pH 3, 5 or 7, with 0, 20, 40 or 60% MeOH	
			μ Bondapak C18	300x4	0.025M NaH_2PO_4 or Na_2HPO_4 buffers of pH 3, 5, 7 or 9, with 0, 20, 40, 60 or 80% MeOH	11, 20
Coc, various opium alkaloids		Identification	Zipax SCX		0.2M NaOH+5% prOH, 1% KNO_3 and 2% ACN (pH 9)	21

Coc, various opium and ergot alkaloids, caffeine, theophylline, quinine, strychnine	Local anaesthetics, various other drugs	Ion-pair chromatography for the separation of drugs of abuse (Table 4.7)	μ Bondapak C18	300x4	0.005M heptanesulfonic acid in MeOH-AcOH-H ₂ O (40:1:59) (pH 3.5)	23
Coc	Local anaesthetics	Identification street drugs	Bondapak Phenyl/Porasil B	1200x3.2	ACN-H ₂ O (85:15) with 0.1% (NH ₄) ₂ CO ₃ by weight	34
Coc, various alkaloids (Table 2.2 and 2.3)	Various drugs	Identification by means of dual wavelength detection	μ Bondapak C18	300x3.9	0.25M NaH ₂ PO ₄ in MeOH-H ₂ O (2:3), pH 7.0	40
			μ Porasil	300x3.9	MeOH-2M NH ₄ OH-1M NH ₄ NO ₃ (27:2:1) CH ₂ Cl ₂ -conc. NH ₄ OH (100:2)	40
Coc, various opium alkaloids, caffeine, quinine, quinidine, strychnine	Local anaesthetics, hypnotics, analgesics	Analysis heroin seizures (Table 7.11)	μ Porasil	300x4	Cyclohexane-(CHCl ₃ -MeOH-NH ₄ OH (800:200:1)) (3:1) ³ conc. NH ₄ OH: 28, 14 or 7%	53
Coc, benzoyllecgonine, cinnamoylcoc, opium alkaloids, caffeine	Local anaesthetics	Analysis illicit cocaine samples (Table 4.6)	Lichrosorb RP2, 5 μ m	150x4.6	MeOH-0.1M NH ₄ NO ₃ (2:3), pH 4.3 with 2M HCl	56
Coc, various opium alkaloids and other alkaloids	Local anaesthetics, amphetamines, barbiturates	Ion-pair HPLC of drugs of forensic interest	μ Bondapak C18, μ Bondapak Phenyl or μ Bondapak CN	300x3.9	0.005M alkylsulfonate (C ₁ , C ₄ , C ₇) in MeOH-H ₂ O-AcOH (40:59:1), (30:69:1) ⁵ (20:79:1), pH 3.5	58, 59, 60
Coc, trans- and cis-cinnamoylcoc, benzoyllecgonine, benzoic acid		Analysis illicit cocaine samples	μ Bondapak C18	300x3.9	MeOH-phosphate buffer (pH 3.0) (1:2)	63
Coc, tropacoc, A, opium alkaloids, various alkaloids	Local anaesthetics, various other drugs	Analysis heroin seizures (Table 7.6)	μ Bondapak C18 or Partisil 10-ODS-3	300x3.9 250x4.6	ACN-H ₂ O-H ₃ PO ₄ (12:87:1) containing 0.02M methanesulfonic acid, pH 2.2	67

*Abbreviations used in Tables 4.8 - 4.11

Coc cocaine
A atropine
S scopolamine

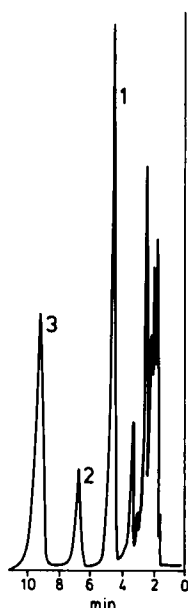


Fig. 4.7. HPLC analysis cocaine and metabolites in urine³¹
 Column Partisil 10-ODS (250x4.6 mm ID), mobile phase 0.25 M potassium dihydrogen phosphate (pH 2.7) containing 17% acetonitrile, flow rate 2 ml/min, detection UV 200 and 235 nm, column temperature 40°C. Peaks: 1, benzoylecgonine; 2, cocaine; 3, benzoylecgonine *n*-ethyl ester (internal standard).

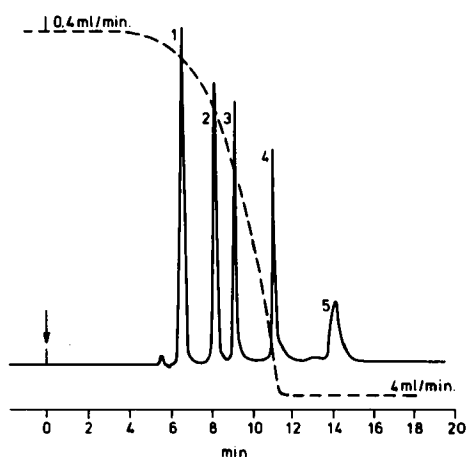


Fig. 4.8. HPLC separation of isomeric cocaine⁴⁹

Column Partisil 10PXS (250x4.6 mm ID), mobile phase heptane - isopropanol - diethylamine (75:25:0.1), flow rate increasing exponentially (see dashed line), detection UV 230 nm. Peaks: 1, *N,N*-dibenzylbenzamide (internal standard); 2, cocaine; 3, allococaine; 4, pseudococaine; 5, allospseudococaine.

Fig. 4.9. Separation of isomeric cocaine⁴⁶

Column Nucleosil C18 5 μ m (150x4.0 mm ID), mobile phase tetrahydrofuran - water (1:4) containing 0.005 M *n*-heptanesulfonic acid and 2% acetic acid, flow rate 1.0 ml/min, detection UV 235 nm. Peaks: 1, cocaine; 2, pseudococaine; 3, allococaine; 4, allospseudococaine. (Reproduced with permission from ref. 46, by courtesy of Recueil des travaux chimiques des Pays-Bas)

Chapter 5

QUINOLINE ALKALOIDS: *CINCHONA* ALKALOIDS

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HPLC has been applied successfully in the analysis of *Cinchona* alkaloids whereby the following problems have been dealt with:

1. Separation of *Cinchona* alkaloids in general.
2. Analysis of such alkaloids present in
 - a) naturally occurring mixtures and in plant material,
 - b) pharmaceutical preparations,
 - c) drugs of abuse,
 - d) food and beverages,
 - e) biological fluids.

Quinidine has, as an antiarrhythmic drug, a narrow therapeutic range, and consequently its quantitative analysis in blood, plasma and serum has been the subject of a series of investigations (Table 5.5). Several authors compared HPLC methods with other methods, such as TLC³¹, GLC^{27,46}, EMIT^{55,56,57} or fluorimetric assays^{18,21,22,24,25,27,28,35,36,38,46,56}. The latter method is the least specific one and it usually gives higher quinidine values because of interference of impurities (dihydroquinidine) and metabolites. The same applies for EMIT. Many of the methods used also determine dihydroquinidine^{14,18,21,25,27,28,29,31,34,35,36,38,46,58,61,65,66,67,74}. The content of dihydroquinidine in quinidine can be as high as 20%. Its determination in quinidine has been described^{6,42,50}. Many quinidine metabolites, some of which have a similar activity to quinidine, have been determined, together with quinidine, by the existing methods^{18,21,23,24,34,35,36,38,46,49,58,61,65,66,67,74}. A possible interference of other drugs in the analysis of quinidine has been reported^{22,25,28,31,35,46,48} and particularly on the interference of quinine^{22,25,27,46,58,67,74}. Weidner et al.³⁵ developed a method that separated quinidine and quinine, using the latter compound as an internal standard.

An eventual interference of quinine in quinidine determinations in biological fluids due to the use of soft drinks containing quinine is unlikely¹⁸.

Bonora et al.^{36,49} described a method for the analysis of quinidine in urine; Rakhit et al.⁶⁵ and Patel⁷⁴ reported methods suited for the analysis of quinidine in urine. Lagerström⁶¹ injected urine directly on the column for the analysis of quinidine. Barrow et al.⁴⁷ reported the analysis of quinine and quinidine and their respective metabolites in rat urine. Flood et al.⁴⁵ determined simultaneously three antiarrhythmic drugs in plasma by means of HPLC. As a common dilutant for drugs of abuse, quinine is often found and determined in mixtures of such drugs (Table 5.1). The analysis of quinine in soft drinks have also been accomplished by means of HPLC (Table 5.6).

Table 5.1

CINCHONA ALKALOIDS IN THE CONTEXT OF HPLC ANALYSIS OF DRUGS OF ABUSE(Chapter 7)

Alkaloids *	Ref	Ref Chapter 7	Alkaloids *	Ref	Ref Chapter 7
Q,Qd	3	6	Q	39	56
Q	4	11	Q,Qd	59	91(Table 7.11)
Q	7	15	Q	60	93
Q	8	18(Fig. 7.2)	Q,Qd	62,63,64	98,99,100
Q	10	21	Q	69	113
Q,Qd	11	22(Table 7.8)	Q	70	117(Fig.7.9)
Q	13	30	Q,Qd	71	118
Q,Qd	19	38(Table 7.3)	Q	73	121(Table 7.6)
Q	26	47			

5.1 ION-EXCHANGE HPLC

Wittwer³ described an ion-exchange separation method for some drugs of abuse. Quinine and quinidine were among the compounds investigated. Murgia and Walton^{9,12} used cation-exchangers loaded with metal ions (Cu^{++} , Zn^{++} , Ni^{++}) to separate a number of alkaloids, including cinchonine and quinine (ligand-exchange chromatography). Twitchett et al.¹³ evaluated a microparticulate cation-exchange column for the analysis of a series of drugs, including quinine. A discussion of the results is given in Chapter 7.

5.2 REVERSED-PHASE HPLC

Johnston et al.⁵⁰ described reversed-phase HPLC of a series of *Cinchona* alkaloids, whereby a chemically bonded octadecyl stationary phase was used and methanol-water-acetic acid (25:75:1) as mobile phase (Fig. 5.1). This mobile phase was introduced by Crouthamel et al.^{18,23,42} for the analysis of quinidine in plasma. Quinidine, dihydroquinidine and 3-hydroxyquinidine could be separated. The solvents mentioned above- in the ratio 20:80:1- were used for the separation of cinchonine and cinchonidine⁵⁰.

Most of the mobile phases employed in the analysis of quinidine in biological fluids are acidic. Drayer et al.^{21,56} and Leroyer et al.^{58,67} separated quinidine and its metabolites by using acetonitrile-acetic acid-water in combination with an octadecyl column (Fig. 5.2). Barrow et al.⁴⁷ used a similar system, but preferred a gradient elution to separate quinine and quinidine from their respective metabolites. Acidic buffers have also been used in mixtures with acetonitrile^{45,48,68,74} (Fig. 5.3).

Powers and Sadee²² preferred a chemically bonded alkyl phenyl stationary phase over an octadecyl phase, because of the poor chromatographic behaviour of the latter type. They used 0.75 M sodium acetate (pH 3.6)-acetonitrile (3:2) as mobile phase.

Because of the short life time of a straight phase column - due to the accumulation of polar substances from the urine on the silica gel used, Bonora et al.³⁶ preferred a reversed-phase separation for the analysis of quinidine in urine. To be able to separate quinidine and its metabolites on an alkyl phenyl column, acetonitrile plus a buffer of pH 4,5 was used as mobile phase. A small amount of tetrahydrofuran added to the mobile phase was found to improve the peak shape and the separation (Fig.5.4). Reece and Peikert⁴⁶ used alkyl phenyl columns for the same kind of analysis (Fig.5.5), but they found that a column temperature of 50°C improved the peak resolution.

*For abbreviations see footnote Table 5.6

The chromatographic system reported by Weidner et al.³⁵ separated quinidine, dihydroquinidine and 3-hydroxyquinidine. Quinine was used as an internal standard, although it had the same retention time as dihydroquinidine, this made it necessary always to carry out two analyses: one with and one without the addition of quinine.

Kline et al.³⁴ used acetonitrile - methanol - 1% aqueous ammonium carbonate (65:31:4) and an octadecyl column to analyze quinidine and dihydroquinidine (Fig.5.6).

Lagerström⁶¹ injected urine directly on an octyl type of column for the analysis of quinidine in urine. The acidic mobile phase, 25% acetonitrile in 0.095 M sodium perchlorate and 0.005 M perchloric acid, allowed fluorescent detection, which solved the problem of solvent peak observed with UV detection. Rakhit et al.⁶⁵ reported the analysis of quinidine in various biological materials (Fig.5.7) using an octyl type of stationary phase.

A cyanopropyl bonded phase has been used for the separation of the four major *Cinchona* alkaloids (quinine, quinidine, cinchonidine, cinchonine) (Fig.5.8)⁷².

Aramaki et al.⁴⁴ performed analysis of basic drugs on a macroporous styrene-divinylbenzene co-polymer (see Chapter 8, Table 8.4).

5.3 ION-PAIR HPLC

Persson and Lagerström^{14,29} analyzed antiarrhythmic drugs in plasma by means of ion-pair partition chromatography. Quinidine and dihydroquinidine were separated on a microparticulate silica gel column loaded by an *in-situ* technique with the aqueous stationary phase: 0.2 M perchloric acid - 0.8 M sodium perchlorate (ca. 35% of the total weight of the silica gel used) (Fig.5.9). The mobile phase, *n*-butanol - dichloromethane - *n*-hexane (1:7:2), was saturated with the stationary phase. A precolumn was used to improve the equilibration of the mobile phase. *n*-Butanol was found to improve the peak symmetry; it lowered, however, the selectivity of the separation of related compounds. Dichloromethane determined the selectivity of the system and *n*-hexane was found to be inert and could be used to decrease the polarity of the mobile phase and - thus - increase the retention times.

With toluenesulfonic acid as pairing-ion, Gaetani et al.¹⁶ analyzed quinidine in blood on a silica gel column. However, quinidine was not separated from its dihydroderivative and also quinine interfered with the analysis.

Lurie^{19,62,63,64,73} described the analysis of some drugs of forensic interest, e.g. quinine, and applied reversed-phase ion-pair chromatography (see Chapter 7).

In a study of ion-pair chromatography with cationic surfactants, which - according to the authors - could be called dynamic ion-exchange chromatography - Terwey-Groen et al.³⁰ presented the analysis of quinine in soft drinks, with an anionic surfactant (dodecylsulfate) as an example (Fig.5.10).

Jeuring et al.⁴³ analyzed quinine in soft drinks. By using dodecylsulfonic acid as pairing-ion, a reversed-phase analysis under acidic conditions became possible. This was necessary for the fluorimetric detection of quinine. The method allowed a direct analysis of quinine in soft drinks without extraction.

5.4 STRAIGHT-PHASE HPLC

Pound and Sears⁶ described a HPLC procedure for a simultaneous analysis of quinine, qui-

nidine, their dihydroderivatives, cinchonine and cinchonidine in commercial preparations. The quantitative determination - with antipyrine as an internal standard - was performed on microparticulate silica gel with tetrahydrofuran - 0.2% ammonia as mobile phase (Fig.5.1). When quinidine and quinine sulfate were analyzed, the percentage of ammonia was increased to 0.4% and 0.5% respectively. Frischkorn and Frischkorn¹⁵ determined quinine in soft drinks by employing methanol - 0.5% ammonia as mobile phase and using a silica gel column. However, quinine and quinidine could not be separated. Achari et al.²⁷ described a similar HPLC method for the analysis of quinidine in human plasma (Fig.5.12).

Kates et al.³¹ used a solvent system consisting of 0.001 M trimethylamine hydrochloride and 0.001 M potassium hydroxide (pH 9) - methanol (1:4) and a microparticulate silica gel column to separate quinidine and dihydroquinidine. Cinchonine was used as internal standard.

Quinidine and dihydroquinidine could also be separated by a method developed by Achari and Theimer²⁰ for the analysis of a series of drugs. However, quinine and quinidine were not separated.

Guentert et al.^{24,38} described a straight-phase separation of quinidine and its major metabolites, extracted from human plasma. On a microparticulate silica gel column and mobile phase hexane - ethanol - ethanolamine (91.5:8.47:0.03) quantitative analysis was performed using primaquine as an internal standard (Fig.5.13). The addition of ethanolamine to the mobile phase eliminated tailing and reduced retention times. Ethanolamine was found to be superior to ammonia because it provides a more stable solvent mixture, enabling automatic injection. The percentage of ethanol could be adjusted in order to alter the retention times. Also the variation of the ethanolamine concentration led to changes in the retention times. However, long equilibration times (1-2 hrs) were needed to achieve stable conditions.

Peat and Jennison²⁵ used a polar mobile phase in combination with a microparticulate silica gel column - as originally described by Jane¹¹ - for the analysis of quinidine in plasma (Fig.5.14). Quinidine and quinine were not, however, separated; cinchonidine was used as internal standard. A similar method was used by Pershing et al.⁶⁶.

A microparticulate silica gel column and a mobile phase of dichloromethane - hexane - methanol - perchloric acid (65:35:5.5:0.1) was applied by Sved et al.²⁸ to determine the quinidine and dihydroquinidine content in plasma. Perchloric acid was added to induce fluorescence, but it also had some influence on the retention times. 3-Hydroxyquinidine was separated from quinidine and dihydroquinidine; however, 2'-quinidinone had the same retention time as quinidine.

Bauer and Untz⁵¹ analyzed a series of *Cinchona* alkaloids by means of straight-phase HPLC (Fig.5.15). They found that the addition of 2.65 ml of water to 1 liter of the mobile phase (chloroform - isopropanol - diethylamine(940:57:1)), which corresponds to about 75% saturation, gave optimum separation, as regards resolution versus time of analysis. To obtain the correct percentage of water in the mobile phase, the water content present in the mixture was determined by the Karl Fischer method, and water was then added to obtain a final concentration of 2.65 ml/l.

The non-aqueous ionic solvents, as described by Flanagan et al.⁷¹ (see Chapter 2) had only limited applicability for quinine because of tailing at low pH. Raising the pH of the ammonium perchlorate solution in methanol to about 9.2 improved the peakshape.

5.5 DETECTION

Due to their strong fluorescence under acidic conditions, *Cinchona* alkaloids have been detec-

ted by means of fluorescence detectors^{16,17,21,28,34,35,43,46,49,56,61,65,66,67} (Fig.5.2, 5.5, 5.6 and 5.7). Usually excitation wavelengths of 330-360 nm have been used, measuring emission at 420-450 nm. However, some authors preferred 245 nm and 340 nm respectively^{21,49,65}. Kline et al.³⁴ described a post-column addition of 1 M sulphuric acid to induce fluorescence of quinidine and dihydroquinidine (Fig.5.6), but acidic mobile phases have mostly been used to allow fluorescence detection^{16,17,21,28,35,43,46,49,56,58,61,65,67}.

According to Weidner et al.³⁵, fluorescence detection is more sensitive than UV detection at 254 nm; however, for routine analysis of quinidine in serum, the additional sensitivity was not found to be necessary. Verpoorte and Baerheim Svendsen⁵ preferred detection at 280 nm over detection at 254 nm. Peat and Jennison²⁵ found about equal responses at 254 and 280 nm; however, the latter wavelength was preferred for UV-detection because of less interference of spurious components in the analysis of quinidine in plasma.

Many authors have preferred detection of quinidine at its maximum at 235 nm^{27,31,36,38,49,72}, but Powers and Sadee²² preferred to detect quinidine at its secondary maximum at 330 nm. Although this meant a five times less sensitivity than a detection at 235 nm, detection at 330 nm was not interfered with by other compounds. For this reason, detection at 325 nm has also been employed⁴⁸.

For the determination of antiarrhythmic drugs in serum, Flood et al.⁴⁵ applied a simultaneous detection at 205 and 254 nm. Bauer and Untz⁵¹ preferred detection at 312 nm, because at this wavelength the 6-methoxy substituted alkaloids have the same extinction coefficient as the non-substituted alkaloids, enabling a direct comparison of the peak areas of the different alkaloids.

Identification of drugs - including quinine - by using the absorbance ratio at 254 and 280 nm was reported by Baker et al.³⁹; Lurie et al.⁷³ used the 220:254 ratio for the same reasons.

Kral and Sontag⁷⁵ employed an electrochemical detector for the analysis of quinine in beverages. The maximum oxidation potential was at -1.55 V vs. SSCE, best results were, however, obtained at -1.4 V. A capacitance-conductance detector for HPLC was developed by Hashimoto et al.³³ and applied to the analysis of alkaloids, e.g. some *Cinchona* alkaloids.

Eckers et al.⁵⁴ reported the application of a coupled LC-MS system to the analysis of *Cinchona* alkaloids.

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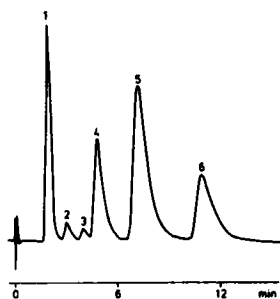


Fig. 5.1. Reversed-phase HPLC separation of some *Cinchona* alkaloids⁵⁰
Column μ Bondapak C18 (300x4 mm ID), mobile phase methanol - water - acetic acid (25:75:1), flow rate 1.5 ml/min, detection UV 254 nm. Peaks: 1, theophylline (internal standard); 2, cinchonine; 3, cinchonidine; 4, quinidine; 5, quinidine and dihydroquinidine; 6, dihydroquinine.

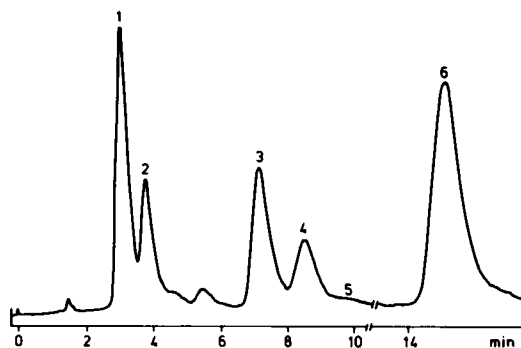


Fig. 5.2. HPLC analysis quinidine and metabolites in serum²¹
Column μ Bondapak C18 (300x4 mm ID), mobile phase 2.5% aqueous acetic acid - acetonitrile (88:12), flow rate 1.8 ml/min, fluorescence detector (excitation 340 nm, emission 418 nm). Peaks: 1, 3-hydroxyquinidine; 2, cupreidine (=desmethylquinidine); 3, quinidine; 4, cinchonidine (internal standard); 5, dihydroquinidine; 6, 2'-quinidinone. (reproduced with permission from ref. 21, by courtesy of the C.V. Mosby Company).

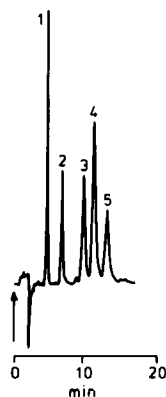


Fig. 5.3. Separation of quinidine and its metabolites⁷⁴
Column Partisil PXS 5/25 ODS (250x4.6 mm ID) with guard column, mobile phase acetonitrile - 1 M sodium dihydrogen phosphate - 1 M sodium perchlorate - 85% phosphoric acid - water (11:2:1:1:85), flow rate 1.5 ml/min, detection UV 254 nm. Peaks: 1, 3-hydroxyquinidine; 2, cupreidine (=desmethylquinidine); 3, quinidine; 4, quinine; 5, dihydroquinidine. (Reproduced with permission from ref. 74, by courtesy of Raven Press)

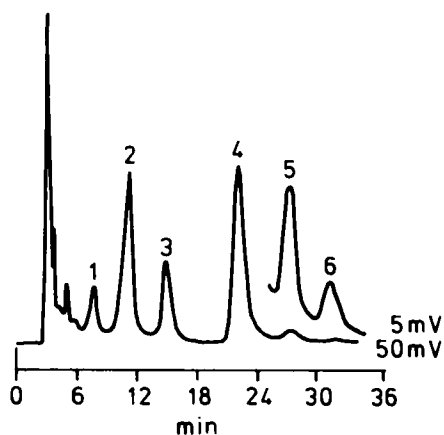


Fig. 5.4. Analysis quinidine and metabolites in urine³⁶

Column μ Bondapak Phenyl (300x3.9 mm ID), mobile phase 0.05 M phosphate buffer (pH 4.5) - acetonitrile - tetrahydrofuran (80:15:5), flow rate 1.16 ml/min, detection UV 230 nm. Peaks: 1, 2'-quinidinone; 2, 3-hydroxyquinidine; 3, oxprenolol (internal standard); 4, quinidine; 5, dihydroquinidine; 6, quinidine N-oxide.

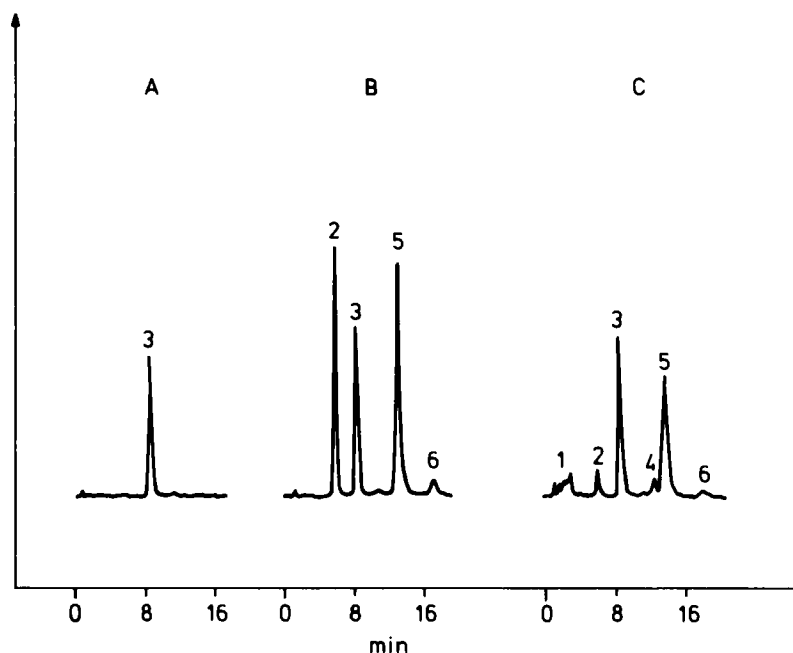


Fig. 5.5. HPLC assay quinidine in plasma⁴⁶

Column μ Bondapak Phenyl (300x4 mm ID), mobile phase 0.0015 M aqueous phosphoric acid - acetonitrile (9:1), flow rate 2 ml/min, fluorescence detection (excitation 320 nm, emission 418 nm). Chromatogram A: blank plasma; chromatogram B: plasma standard (10 μ mole/l quinidine, 5 μ mole/l 3-hydroxyquinidine); chromatogram C: plasma from patient on chronic oral quinidine therapy. Peaks: 1, polar metabolites; 2, 3-hydroxyquinidine; 3, internal standard (cinchonidine); 4, unidentified metabolite; 5, quinidine; 6, dihydroquinidine.

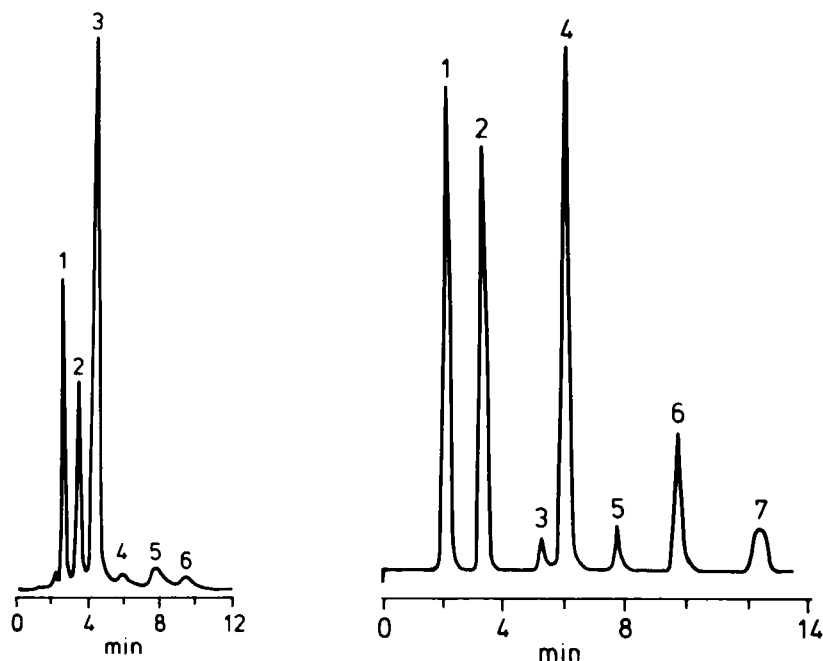


Fig. 5.6. Analysis quinidine and metabolites in plasma³⁴
 Column μ Bondapak C18 (300x3.9 mm ID), mobile phase acetonitrile - methanol - 1% aqueous ammonium carbonate (65:31:4), flow rate 3.5 ml/min, detection with a fluorimeter after post-column addition of sulfuric acid (excitation 350 nm, emission 450 nm). Peaks: 1, 3-hydroxyquinidine; 3, quinidine; 6, dihydroquinidine; 2, 4 and 5 unidentified metabolites. Limit of sensitivity 50 ng/ml plasma. (Reproduced with permission from ref. 34, by courtesy of the American Chemical Society).

Fig. 5.7. Separation of quinidine and some of its metabolites⁶⁵
 Column Ultrasphere C8 (150x4.6 mm ID), mobile phase acetonitrile - methanol - tetrahydrofuran - 0.01 M aqueous triethylamine (pH 2.5 with phosphoric acid) (5:5:3:87), flow rate 1.0 ml/min, fluorescence detection (excitation 245 nm, emission 435 nm). Peaks: 1, quinidine-10,11-dihydrodiol; 2, 3-hydroxyquinidine; 3, quinidine N-oxide; 4, quinidine; 5, dihydroquinidine; 6, 3-methyl-5-triazolophthalazine (internal standard); 7, 2'-quinidinone. (Reproduced with permission from ref. 65, by the courtesy of Clinical Chemistry)

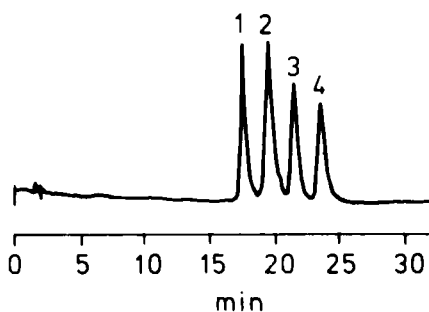


Fig. 5.8. Separation of major Cinchona alkaloids⁷²
 Column Spherisorb CN 5 μ m (250x4.6 mm ID), mobile phase acetonitrile - methanol - tetrahydrofuran - 0.0068 M phosphoric acid (pH 7.0 with 1 M sodium hydroxide), flow rate 1.5 ml/min, temperature 50°C, detection UV 231 nm. Peaks: 1, quinine; 2, quinidine; 3, cinchonidine; 4, cinchonine.

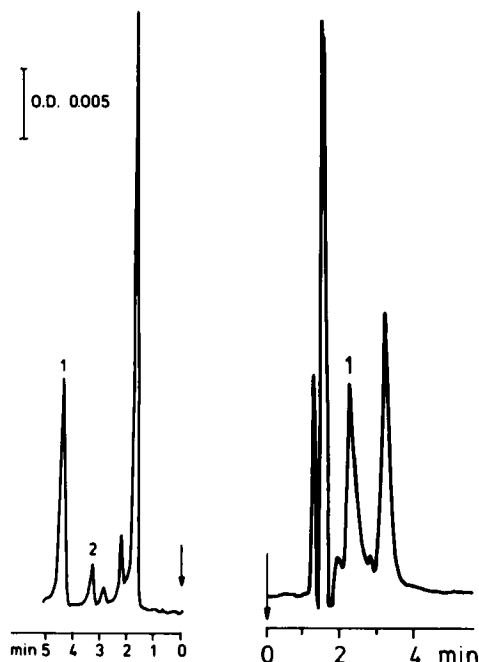


Fig. 5.9. Analysis quinidine and dihydroquinidine in plasma^{14,29}
Column Lichrosorb Si100 10 μ m coated with ca. 35% 0.2 M perchloric acid and 0.8 M sodium perchlorate (150x4.5 mm ID), mobile phase n-butanol - dichloromethane - n-hexane (1:7:2) saturated with the stationary phase, detection UV 254 nm. Peaks: 1, quinidine; 2, dihydroquinidine.

Fig. 5.10. Analysis quinine in lemon tonic³⁰
Column Lichrosorb RP8 10 μ m (150x3 mm ID), mobile phase 25% propanol and 0.01% sodium dodecylsulfate in 0.025 M sodium sulfate (pH 6.4), detection UV 243 nm. Peak: 1, quinine. 10 μ l injection of mixture of 1 ml tonic diluted with 2 ml eluent.

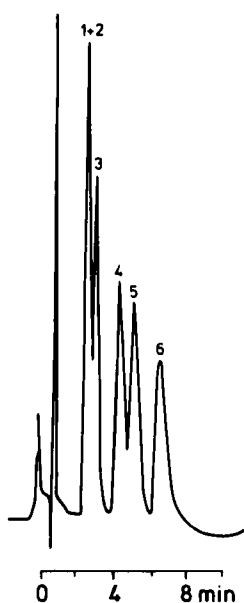


Fig. 5.11. Separation of some Cinchona alkaloids⁶
Colum Lichrosorb Si60 10 μ m (250x1.8 mm ID), mobile phase tetrahydrofuran - 0.2% concentrated ammonia, flow rate 1 ml/min, detection UV 254 nm. Peaks: 1, cinchonine and cinchonidine; 2, quinidine; 3, quinine; 4, dihydroquinidine; 5, dihydroquinine. (Reproduced with permission from ref. 6, by the courtesy of the Canadian Pharmaceutical Association)

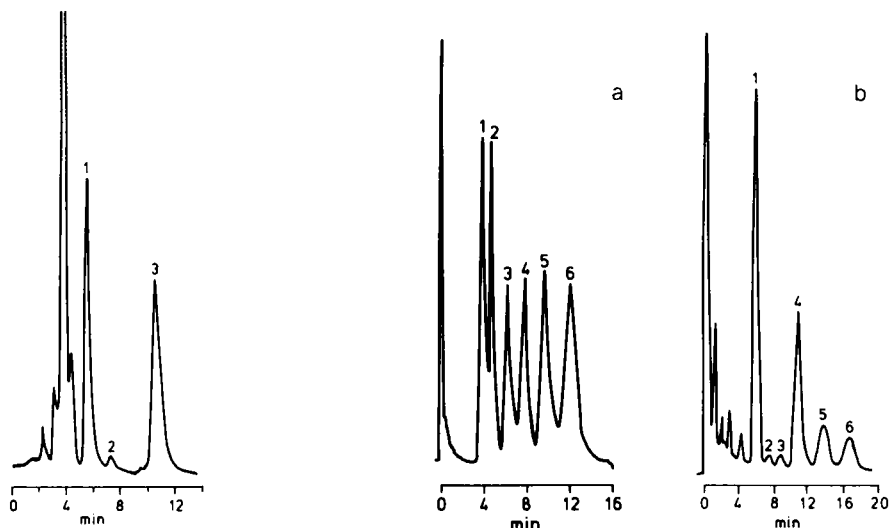


Fig. 5.12. Analysis quinidine in plasma²⁷

Column Partisil 10 μm (250x4.6 mm ID), mobile phase methanol - 0.75% concentrated ammonia, flow rate 1.1 ml/min, detection UV 236 nm. Peaks: 1, quinidine; 2, dihydroquinidine; 3, strychnine (internal standard). (Reproduced with permission from ref. 27, by courtesy of Journal Chromatographic Science).

Fig. 5.13A. Separation of quinidine and metabolites in a test mixture^{24,38}

Column Micropak S110 (250x2.1 mm ID), mobile phase hexanes - ethanol - ethanolamine (91.5:8.47:0.03), flow rate 1 ml/min, detection UV 235 nm. Peaks: 1, quinidine; 2, dihydroquinidine; 3, 2'-quinidinone; 4, primaquine; 5, quinidine-N-oxide; 6, 3-hydroxyquinidine.

Fig. 5.13B. Conditions and peak numbering as in Fig. 5.13A, except for the ratio of the mobile phase, which is (92.97:7.0:0.03). Analysis in plasma.

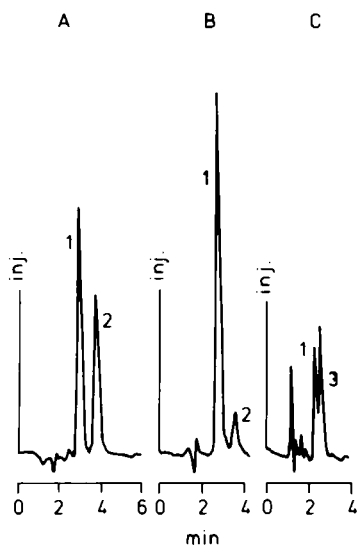


Fig. 5.14. Analysis quinidine and dihydroquinidine in plasma²⁵
Column Partisil 10 μm (250x4.6 mm ID), mobile phase methanol - 1 M ammonium nitrate - 2 M ammonia (27:2:1), flow rate 1.2 ml/min, detection UV 280 nm. Peaks: 1, Quinidine; 2, dihydroquinidine; 3, cinchonidine. (reproduced with permission from ref. 25, by the courtesy of Clinical Chemistry)

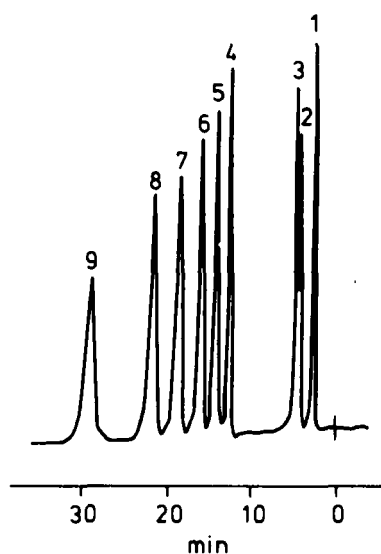


Fig. 5.15. Separation Cinchona alkaloids⁵¹
 Column Lichrosorb Si60 5 μm (150x4.6 mm ID), mobile phase chloroform - isopropanol - diethylamine - water (940:57:1:2.65), flow rate 1.2 ml/min, detection UV 312 nm. Peaks: 1, quinidine; 2, epiquinidine; 3, epiquinine; 4, quinidine; 5, cinchonine; 6, cinchonidine; 7, quinine; 8, dihydroquinidine; 9, dihydroquinine.

TABLE 5.2

HPLC ANALYSIS OF VARIOUS COMPOUNDS INCLUDING CINCHONA ALKALOIDS

ALKALOIDS*	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID mm	MOBILE PHASE	REF.
Q,C,opium alka- loids,cocaine, caffeine		Separation by means of dyna- mic coating HPLC	Corasil I and II, dynamically coated with Poly G-300 (2%)	1000x1	Heptane-EtOH(10:1) with different percentage of saturation with the stationa- ry phase Poly G-300	1,2
Q,Qd,C,Cd,20 other alkaloids		Analysis alkaloids	Merckosorb Si60,5 µm	300x2	CHCl ₃ -MeOH(9:1),(8:2),(7:3) Et ₂ O-MeOH(8:2),(7:3),(6:4)	5
Q,C,opium-, tropane alkaloids strychnine,nico- tine		Separation on ion-exchange resins (ligand-exchange LC)	Hydrolyzed Poragel PT, loaded with Cu ²⁺ Bio-Rad PC20,loaded with Cu ²⁺	470x6.3 470x6.3	0.06M NH ₄ OH in 33% EtOH 0.2M NH ₄ OH in 33% EtOH 0.05M NH ₄ OH in 33% EtOH 0.03M NH ₄ OH in 33% EtOH	9,12
C,opium alkaloids		Quantitative analysis opium alkaloids	Spherisorb silica,5µm	250x3	CHCl ₃ -MeOH-NH ₄ OH(81.5:18:0.5)	32
Q,Qd,C,brucine, strychnine, eme- tine, reserpine, yohimbine,scopo- lamine		Detection with conductance detector	Silica gel 10 µm		CHCl ₃ -MeOH-hexane (7:3:10)	33
Cd,codeine,nar- ceine,brucine, colchicine,aco- nitine,caffeine	Santonine	Effect solvent composition on retention	Lichrosorb RP2, 10 µm	120x3.5	MeOH-H ₂ O (1:4),(2:3),(3:2) (4:1) MeOH	41
Q,C,atropine, acridine,ephe- drine,opium al- kaloids, reser- pine,strychnine, yohimbine		Separation on styrene-divinyl- benzene polymer (Table 8.4)	Hitachi gel 3010, 10 µm	220x4.6	ACN-0.02M NH ₄ OH(3:2) ACN-0.02M tetrabutyl- ammoniumhydroxide (3:7), (3:2)	44
Qd,atropine,sco- polamine,caffeine, codeine,papaverine, ephedrine	Various drugs	Retention behaviour basic drugs in ion-pair HPLC	µBondapak C18 µBondapak Phenyl µBondapak CN µBondagel Chromegabond C8 Chromegabond C ₆ H ₁₁	300x4	0.005M heptanesulfonic acid in H ₂ O-MeOH-AcOH(50:49:1) pH 4.0)	53
Q, various al- kaloids	Flurazepam,va- rious drugs	Separation basic drugs with non-aqueous ionic solvents	Spherisorb S5W silica	250x4.9	MeOH-HClO ₄ -conc.NH ₄ OH(1000:3.55; 9.2)(pH 9.2)	71

*For abbreviations see footnote Table 5.6

TABLE 5.3

HPLC ANALYSIS CINCHONA ALKALOIDS IN PLANT MATERIAL OR NATURALLY OCCURRING MIXTURES

ALKALOIDS *	AIMS	STATIONARY PHASE	COLUMN DIM LxID mm	MOBILE PHASE	REF.
Q,Qd,C,Cd,HQ,HQd	Separation and determination	Lichrosorb Si60 10 μ m	250x1.8	THF-25% NH_4OH (99.8:0.2),(99.6:0.4), (99.5:0.5)	6
Q,Qd,C,Cd,HQ,HQd	Separation and determination	μ Bondapak C18	300x4	MeOH-H ₂ O-AcOH(25:75:1),(20:80:1)	50
Q,Qd,C,Cd,HQ,HQd, epiQ,epiQd,quinidinone	Separation (Fig.5.15)	Lichrosorb Si60 5 μ m	150x4.6	CHCl_3 -isoPrOH-H ₂ O-DEA(940:57:2.65:1)	51
Q,C,HQ	Coupling LC-MS	Partisil 5 μ m	not given	CHCl_3 -ACN-aq. NH_4OH (75:24.5:0.5)	54
Q,Qd,C,Cd	Separation alkaloids from plant material or cell cul- tures (Fig. 5.8)	Spherisorb CN	250x4.6	ACN-MeOH-THF-0.0068 M phosphate buffer(pH 7)(17:28.7:3.3:50)	72

TABLE 5.4

HPLC ANALYSIS CINCHONA ALKALOIDS IN PHARMACEUTICAL PREPARATIONS

ALKALOIDS *	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID mm	MOBILE PHASE	REF.
Q,Qd,C,Cd,HQ, HQd	Antipyrine	Determination in pharmaceu- tical preparations (Fig.5.11)	Lichrosorb Si60 10 μ m	250x1.8	THF-25% NH_4OH (99.8:0.2), (99.6:0.4)(99.5:0.5)	6
Q,Qd,HQd,xanthi- nes,strychnine, tropane alkaloids, codeine,papaver- ine,ephedrine	Various drugs	Analysis of pharmaceuticals	Partisil 10 μ m	250x4.6	CH_2Cl_2 -MeOH(1:3) with 1% 29% NH_4OH	20
Q,opium-,trop- ane alkaloids, strychnine,caf- feine,aconitine, emetine,cephael- ine	Sulfanilamide, phenytoine,pheno- barbital	Identification in pharma- ceuticals (Fig.7.14)	Partisil PXS 5/25	250x4.6	Et_2O sat with 50-100% H_2O + 0.05-0.8% DEA	40
Qd,HQd	Theobromine	Purity control Qd, raw material,and pharmaceuticals	μ Bondapak C18	300x3.9	MeOH-H ₂ O-AcOH(25:71:4)	42

*For abbreviations see footnote Table 5.6

Q,Qd,C,Cd,HQ,HQd Theophylline	Purity control,Q and Qd, raw materials, and pharmaceuticals (Fig.5.1)	μ Bondapak C18	300x4	MeOH-H ₂ O-AcOH(25:75:1), (20:80:1)	50
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TABLE 5.5

HPLC ANALYSIS CINCHONA ALKALOIDS IN BIOLOGICAL MATERIAL

ALKALOIDS *	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID mm	MOBILE PHASE	REF.
Qd,HQd		Determination in plasma, using ion-pair partition HPLC (Fig.5.9)	Lichrosorb Si100, 10 μ m coated with 35% 0.2M HClO ₄ +0.8M NaClO ₄	150 or 200x4.5	n-BuOH-CH ₂ Cl ₂ -n-hexane (1:7:2)sat. with the stationary phase	14,29
Qd,Q,HQd		Determination in blood	Lichrosorb Si60	250x2	0.02M toluenesulfonic acid - 0.05M(NH ₄)H ₂ PO ₄ (pH 3)+40% MeOH	16
Qd,HQd		Determination in blood	Phenyl/Corasil	1000x2.5	Gradient elution A 0.05M H ₂ PO ₄ containing 0.5M (NH ₄) ₂ SO ₄ B MeOH gradient:100% A to A:B (67:33)	17
Qd,HQd,theo-bromine, 3OHQd		Determination in serum	μ Bondapak C18	300x4	MeOH-H ₂ O-AcOH(25:71:4) (pH 2.6)	18,23
Qd,HQd,Cd, 3OHQd,Cud, 2'-quinidinone		Determination in serum (Fig.5.2)	μ Bondapak C18	300x4	ACN-2.5% aq. AcOH(12:88)	21,56
Qd,Q,2'-quinidinone,HQd	Various drugs	Determination in serum	μ Bondapak Phenyl	300x3.9	ACN-0.75M NaOAc buffer (pH 3.6) (2:3)	22
Qd,HQd,3OHQd, 2'-quinidinone Qd N-oxide , primaquine		Determination in plasma (Fig.5.13)	Mikropak Si 10	250x2.1	Hexanes-EtOH-ethanolamine (91.5:8.47:0.03), (92.97:7.0:0.03)	24,38
Qd,Cd,HQd,Q	Various drugs	Determination in plasma (Fig.5.14)	Partisil 10	250x4.6	MeOH-1M NH ₄ NO ₃ -2M NH ₄ OH (27:2:1)	25
Qd,HQd,2'-quinidinone,strychnine		Determination in plasma (Fig.5.12)	Partisil 10	250x4.6	MeOH-conc. NH ₄ OH (99.25:0.75)	27

*For abbreviations see footnote Table 5.6

Qd,HQd,2'-quini- dinone,30HQd		Determination in plasma, using ion-pair extraction	Lichrosorb Si60, 5 μ m	250x3	CH ₂ Cl ₂ -hexane-MeOH-70% HClO ₄ (60:35:5.5:0.1)	28
Qd,HQd,C		Determination in plasma	Partisil 10SCX	250x4.6	MeOH-0.001M trimethylamine and 0.001M KOH in H ₂ O (pH ca 9)(4:1)	31
Qd,HQd,30HQd		Determination in plasma (Fig.5.6)	μ Bondapak C18	300x3.9	ACN-methanol-1% aq.(NH ₄) CO ₃ (65:31:4)	34
Qd,HQd,Q,30HQd, 2'-quinidinone		Determination in serum	Micropak MCH-10	250x2.1	0.01M KH ₂ PO ₄ in H ₂ O contain- ing 0.85% H ₃ PO ₄ and 10% MeOH	35
Qd,HQd,30HQd, 2'-quinidinone, Qd N-oxide	Oxprenolol	Determination in urine (Fig.5.4)	μ Bondapak Phenyl	300x3.9	ACN-THF-0.05M phosphate buffer(pH 4.5)(15:5:80)	36
Q,xanthine alka- loids	Various drugs	Identification in serum	Partisil-10 ODS	250x4.6	MeOH-0.049M H ₃ PO ₄ ,0.049M KH ₂ PO ₄ buffer (pH 2.15) (2:3)	37
Qd	Disopyramide, lidocaine p-chlorodiso- pyramide	Determination in serum	μ Bondapak C18	300x4	ACN-0.03M KH ₂ PO ₄ buffer (pH 4.45)(28:72)	45
Qd,HQd,30HQd, Cd,Q	Disopyramide, procainamide, N-acetylproca- inamide	Determination in plasma (Fig.5.5)	μ Bondapak Phenyl	300x4	ACN-0.0015M aq H ₃ PO ₄ (1:9)	46
Q,Qd,HQ,HQd,Cu, Cud,30HQd,OHQ, Q-10,11dihydrodiol Qd-10,11dihydro- diol,2'-quini- dinone,2'-quini- none		Separation and isolation Q and Qd metabolites from rat urine	μ Bondapak C18	300x3.9 300x7.8	Gradient elution A H ₂ O-AcOH (99:1) B H ₂ O-ACN-AcOH(40:59:1) gradient A:B(9:1) to (15:85) gradient A:B(9:1)to(2:8), solvent B contained 0.1% THF	47
Qd	Disopyramide,pro- cainamide,tocai- nide,lignocaine, p-chlorodisopyra- mide	Determination in plasma	Lichrosorb RP8, 10 μ m	250x4.6	ACN-0.05M phosphate buffer (pH 3.0)(27:73)	48
Qd,HQd,30HQd, 2'-quinidinone, Qd N-oxide	Pronethanol	Determination in plasma and urine	μ Bondapak Phenyl	300x3.9	ACN-THF-0.05M phosphate buffer (pH 4.75)(15:5:80)	49

Qd,HQd,7'-tri-fluoromethyl-di-hydrocinchonidine	Determination in plasma and urine	Partisil, 10 μ m	250x4.6	CH ₂ Cl ₂ -MeOH-conc.NH ₄ OH (95.5:4:0.5)	52
Q,Qd,HQd,30HQd	Determination in plasma	μ Bondapak C18	300x3.9	ACN-AcOH-H ₂ O(10:4:86)	58,67
Qd,HQd,30HQd	Direct analysis of drugs in urine	Lichrosorb RP8	150x4.5	ACN-0.095M NaClO ₄ ,0.005M HClO ₄ (1:3)	61
Qd,HQd,30HQd, 2'-quinidinone, Qd N-oxide, Qd 10,11-dihydro-diol	Determination in plasma, urine and bile (Fig.5.7)	Ultrasphere C8	150x4.6	ACN-MeOH-THF-0.01M TrEA (5:5:3:87)(pH 2.5 with H ₃ PO ₄)	65
Qd,HQd,30HQd, 2'-quinidinone, Cud,primaquine	Determination in plasma	Partisil 10	250x4.5	MeOH-1M NH ₄ NO ₃ -2M NH ₄ OH (28:1:1)	66
Qd Disopyramide,mono-dealkylated disopyramide	Determination in plasma	Spheri-5 RP8	not specified	ACN-0.05M NaH ₂ PO ₄ buffer (pH 3.00)(2:3)	68
Q,Qd,HQd, 30HQd,Cud	Determination in serum and urine (Fig.5.3)	Partisil PXS 5/25 ODS	250x4.6	ACN-1M NaH ₂ PO ₄ -1M NaClO ₄ -85% H ₃ PO ₄ -H ₂ O(11:2:1:1:85)	74

TABLE 5.6

HPLC ANALYSIS CINCHONA ALKALOIDS IN FOOD AND BEVERAGES

ALKALOIDS *	AIMS	STATIONARY PHASE	COLUMN DIM. LxID mm	MOBILE PHASE	REF.
Q	Determination in soft drinks and aq. solutions	Spherosil 5 μ m	250x3	MeOH-25% NH ₄ OH(99.5:0.5)	15
Q	Analysis in soft drink (Fig.5.10)	Lichrosorb RP8, 10 μ m	150x3	25% PrOH,0.01% Na-dodecylsulfate in 0.025M Na ₂ SO ₄	30
Q	Analysis in soft drinks	Lichrosorb RP8, 10 μ m	250x4.6	0.005M Na-dodecyl sulfonate ACN-H ₂ O(3:1),HClO ₄ added to pH 3	43
Q	Analysis in soft drinks, using electrochemical detection	Nucleosil 5C18	250x4	MeOH- 1M NaNO ₃ -1M citric acid - 1M Na-citrate - H ₂ O (64:10:2:3:21)(pH 6)	75

* Abbreviations used:

C=cinchonine, Cd=cinchonidine, Q=quinine, Qd=quinidine, H-=dihydro-

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Chapter 6

PHENYLETHYLAMINES AND ISOQUINOLINE ALKALOIDS

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The isoquinoline alkaloids form a large group of compounds comprising a variety of chemical structures. HPLC methods have so far been applied only for a few of them, i.e. mescaline - as a drug of abuse (Table 6.1) - emetine and cephaeline, as well as berberine. The opium alkaloids have been dealt with separately (see Chapter 7).

6.1. ION-EXCHANGE HPLC

Walton³ studied the analysis of alkaloids on ion-exchange materials loaded with metal ions (see Chapter 7). McMurtrey et al.²⁶ reported on the analysis of some dopamine derived isoquinoline alkaloids by means of ion-exchange HPLC. Three different commercially available cation-exchange materials were compared. The effect of the ionic strength, pH and organic modifiers in the mobile phase were investigated. It was found that not only ion-exchange mechanisms were involved in the retention of the alkaloids. The effect of the pH of the mobile phase on the retention was minimal; however, above pH 5.5 an increased tailing was observed. Addition of organic modifiers decreased the elution time in the order *n*-butanol - dioxane - isopropanol - acetonitrile - ethanol. Increased temperature reduced the retention times of the alkaloids and increased column efficiency. Partisil as stationary phase differed in several aspects from the other two stationary phases tested. Some representative separations are presented in (Fig.6.1 and 6.2).

The analysis of berberine in crude drugs by means of pellicular cation-exchange columns have been reported by Akada et al.^{10,13}.

6.2. REVERSED-PHASE HPLC

Quantitative analysis of the quaternary alkaloid α -tubocurarine in curare has been performed on an octadecyl bonded stationary phase⁹. The influence of pH, buffer concentration, and the nature of the cation in the mobile phase were investigated. Optimum pH was found to be 4. As the cation in the mobile phase, tetramethylammonium gave better separation than potassium or ammonium. The concentration of the buffer did not affect the retention of the alkaloids; however, a higher concentration gave improved peak shape. To reduce the time of analysis a gradient elution was preferred (Fig.6.3).

Berberine - also a quaternary alkaloid - has been analyzed on an octadecyl column using acetonitrile - phosphate buffer (pH 5.2) (3:2) as mobile phase¹¹.

Separation of Amaryllidaceae alkaloids has been achieved on an octyl column with methanol - water (3:2) containing traces of ammonia³¹. Gfeller et al.²⁰ described the analysis of some

TABLE 6.1

ISOQUINOLINE ALKALOIDS IN THE CONTEXT OF HPLC ANALYSIS OF DRUGS OF ABUSE (CHAPTER 7)

Alkaloids	Ref.	Ref. in Chapter 7
Mescaline	1	11
Tubocurarine	4	21
Tubocurarine	7	30
Mescaline	19	56
Cephaeline	23	61
Mescaline	34,35,36	98,99,100

alkaloids - including emetine - on a chemically bonded diol stationary phase. It allowed the use of an exclusively aqueous phase - desirable for a post-column fluorimetric ion-pair reactor. Davis²⁴ analyzed glaucine and its metabolite dehydroglaucine in microbial cultures. For the quantitative analysis on a phenyl-type of bonded phase, papaverine was used as internal standard. The acetonitrile content of the mobile phase (acetonitrile - methanol - 0.05 M potassium dihydrogenphosphate (2:4:5)) was found to be critical for the resolution of the alkaloids and for the peakshape.

DeBros and Gissen¹⁷ analyzed tubocurarine on an octadecyl column using acetonitrile - water (18:82) containing 0.2 M perchloric acid (pH 5.4) as mobile phase.

6.3. ION-PAIR HPLC

Bannister et al.²¹ determined emetine in plasma with the aid of HPLC. After extraction from the biological fluid with dichloromethane the alkaloid was oxidized with mercuric acetate, yielding a fluorescent product. Several alkylsulfonates were tested as the pairing ion for both emetine and its oxidation product. An increased alkyl chain length was found to improve the peak shape. For emetine a mobile phase consisting of 0.0025 M octanesulfonate and 0.5% acetic acid in methanol - water (56:44) was found suitable, in combination with an octadecyl column. For the more polar oxidation product the ratio methanol - water was changed to 3:2.

Several investigators analyzed quaternary protoberberine alkaloids by means of reversed-phase ion-pair HPLC^{28,30,39}. Hashimoto et al.³⁰ reported the use of micromanipulators to transfer the alkaloids of a plant cell to the HPLC system. The alkaloids were subsequently separated on an octyl bonded stationary phase with the mobile phase acetonitrile - tetrahydrofuran - 0.1 N tartaric acid - sodium dodecylsulfate (20:20:59.5:0.5). A similar solvent has been employed for the analysis of berberine, palmatine and coptisine in plant material, though the tetrahydrofuran was changed for methanol and the ratio of the solvent was different³⁹.

Meulemans et al.³³ determined tubocurarine in plasma with the aid of an HPLC system consisting of an octadecyl column in combination with the mobile phase methanol - water (2:3) containing triethylamine, phosphoric acid and pentanesulfonic acid (pH 3.4). Parkin³² used tubocurarine as internal standard in the analysis of alcuronium in plasma. The alkaloids were separated on an octadecyl bonded phase with the mobile phase methanol - water (4:1) containing 0.25% acetic acid and 0.005 M dodecylsulfate.

6.4. STRAIGHT-PHASE HPLC

Emetine and cephaeline can be analyzed on silica gel with chloroform - methanol as mobile

TABLE 6.2

LIQUID CHROMATOGRAPHIC DATA FOR CACTACEAE ALKALOIDS AND RELATED COMPOUNDS¹⁴.

Column Lichrosorb Si60, 10 μ m (300x4.5 mm ID) in series with μ Porasil, 8 μ m (300x4.5 mm ID), mobile phase S1 acetonitrile - conc. ammonia (96:4), flow rate 2.0 ml/min and S2 chloroform - 1% conc. ammonia in methanol (9:1), flow rate 1.0 ml/min, detection UV 254 nm.

Alkaloid	Retention time (min) and k'			
	S1		S2	
	t _r	k'	t _r	k'
<i>Phenethylamines</i>				
3-Hydroxy-4-methoxyphenethylamine	23.2	6.48	-**	-
4-hydroxy-3-methoxyphenethylamine	14.4	3.64	-**	-
<i>Tetrahydroisoquinolines</i>				
Salsoline (6-hydroxy-7-methoxy-1-methyl-)	15.0	3.69	-**	-
Isosalsoline (7-hydroxy-6-methoxy-1-methyl-)	17.5	4.47	-**	-
Arizonine (8-hydroxy-7-methoxy-1-methyl-)	11.7	2.66	-**	-
O-Methylcorypalline (N-methyl-6,7-dimethoxy-)	5.2	0.63	11.1	0.79
N-Methyl-7,8-dimethoxy-1,2,3,4-tetrahydroisoquinoline	3.9	0.22	8.8	0.42
N-methylsalsoline *	7.7	1.31	33.9	4.38
N-methylisosalsoline *	7.6	1.48	31.8	4.00
Hydrocotarnine (N-methyl-8-methoxy-6,7-methylenedioxy-)	3.9	0.22	9.0	0.45
N-Methyl-6-methoxy-7,8-methylenedioxy-1,2,3,4-tetrahydroisoquinoline	3.9	0.22	8.0	0.3
Pellotine (1,2-dimethyl-8-hydroxy-6,7-dimethoxy-)	7.4	1.31	29.0	3.60
Gigantine (1,2-dimethyl-5-hydroxy-6,7-dimethoxy-)	7.1	1.22	22.8	2.70
6,7-Dimethoxy-1,2,3,4-tetrahydroisoquinoline	10.2	2.19	41.5	5.59
Salsolidine (6,7-dimethoxy-1-methyl-)	8.3	1.59	37.4	4.94
Carnegine (1,2-dimethyl-6,7-dimethoxy-)	5.4	0.69	17.8	1.82

* not identified in cacti

** t_r > 40 min

phase^{2,37}. With the addition of ammonia³⁸ or diethylamine⁴⁰ this solvent system has been used for the analysis of these alkaloids in Ipecac plant material. Frei et al.^{5,6,18} reported the analysis of some alkaloids including emetine and cephaeline as dansyl derivatives on silica gel (Fig.7.15).

A series of isoquinoline alkaloids and phenylethylamine derivatives from Cactaceae has been analyzed on silica gel¹⁴ (Table 6.2). Addition of alkali to the mobile phase was found to reduce tailing. The system described could also be used for semipreparative purposes.

Mescaline, as well as a series of other stimulant drugs with a phenylethylamine structure, has been analyzed as β -napthoquinone-4-sulfonate derivatives on a silica gel column. In the analysis of urine, interfering peaks were not observed²⁵. The anti-tumor alkaloid thalicarpine and some related alkaloids could be separated on silica gel¹⁵ (Fig.6.4) after extraction from urine.

6.5. DETECTION

Isoquinoline alkaloids are usually detected at 254 and 280 nm with sufficient sensitivity. However to increase the sensitivity of the analysis of tubocurarine in plasma, deBros and Gissen¹⁷ preferred 204 nm above 280 nm, because of the tenfold enhancement of the UV-absorption at the former wavelength. To improve the sensitivity and the selectivity of the detection of some alkaloids, Frei et al.^{5,6,18} prepared dansyl derivatives. Emetine and cephaeline coupled with, respectively, one and two dansyl groups enabled fluorimetric detection (see Chapter 7 for reaction conditions, and Fig.7.15).

Post-column fluorescent ion-pair formation has been used to improve the sensitivity and selectivity of the detection, i.e., of emetine²⁰ (Chapter 4).

A more than 50-fold increase in sensitivity in the analysis of emetine in plasma was achieved by precolumn oxidation of the alkaloid with 1% mercuric acetate in acetic acid - 35 % aqueous sodium hydroxide - 96 % ethanol (73:27:900) at room temperature and with one hour reaction time, in combination with fluorimetric detection (excitation 225 nm, emission 418 nm)²¹. The fluorescent protoberberine alkaloids have been detected fluorimetrically using excitation at 350 nm and measuring emission at 520 nm³⁹.

Endo et al.²⁵ reported a precolumn derivatization method with sodium β -napthoquinone-4-sulfonate for stimulant amines. Detection of the coloured derivatives at 450 nm increased the sensitivity about 25-fold, when compared with UV-detection of the underivatized compounds. UV detection of the coloured derivatives at 245 and 280 nm was also more sensitive - 2.4 and 3.7-fold increase in sensitivity respectively. However, interfering peaks made it less suitable for the analysis of urine extracts.

The ratio of UV-absorbance at 245 and 280 nm has been used as a further characteristic feature in combination with the retention times for the identification of various drugs - including mescaline¹⁹.

An electrochemical detection method has been applied to the analysis of some isoquinoline alkaloids^{16,26}. McMurtrey et al.²⁶ found the method about 1000 times more sensitive than UV detection at 280 nm.

Selective detection of Amaryllidaceae alkaloids in the low μ g range with a circular dichroism spectrophotometer has been described by Westwood et al.³¹. The method offers a high degree of selectivity for optically active alkaloids with suitable chromophores, since the

wavelength of detection can be varied. CD-active compounds can be detected in complex mixtures of other compounds without interference.

Coupling of LC with mass-spectrometry has been reported for the analysis of Amaryllidaceae alkaloids²⁹.

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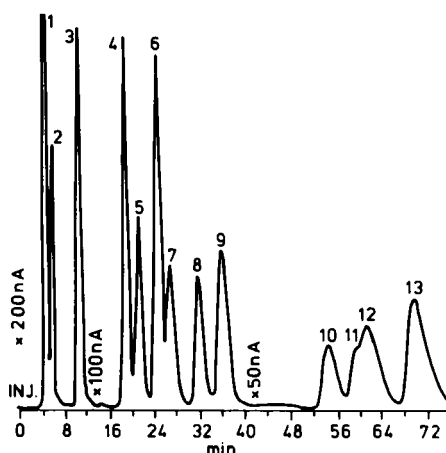
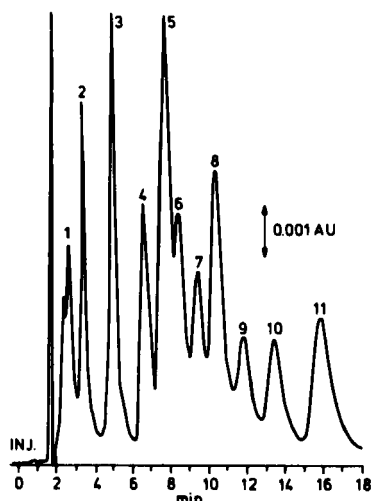


Fig. 6.1. Separation of some dopamine derived isoquinoline alkaloids²⁶
Column Nucleosil SA 10 μ m (250x3.2 mm ID), mobile phase 0.5 M $\text{NH}_4\text{H}_2\text{PO}_4$ + 5% n-butanol, flow rate 1.0 ml/min, detection UV 280 nm. Peaks: 1, dopamine; 2, salsolinol; 3, tetrahydropapaveroline; 4, 2,3,10,11-tetrahydroxyberbine; 5, 2,3,9,10-tetrahydroxyberbine and 3'-O-methyltetrahydropapaveroline; 6, 7-O-methyltetrahydropapaveroline; 7, 4'-O-methyltetrahydropapaveroline; 8, 6-O-methyltetrahydropapaveroline; 9, 2-O-methyltetrahydroxyberbine; 10, 11-O-methyltetrahydroxyberbine and 10-O-methyltetrahydroxyberbine.

Fig. 6.2. Separation of some dopamine derived isoquinoline alkaloids²⁶
Column Vydac TP 401 SCX 10 μ m (250x3.2 mm ID), mobile phase 0.2 M $\text{NH}_4\text{H}_2\text{PO}_4$, flow rate 0.5 ml/min, column temperature 51°C, detection UV 280 nm. Peaks: 1, dopamine; 2, salsolinol; 3, tetrahydropapaveroline; 4, 2,3,10,11-tetrahydroxyberbine; 5, 3'-O-methyltetrahydropapaveroline; 6, 2,3,9,10-tetrahydroxyberbine; 7, 7-O-methyltetrahydropapaveroline; 8, 4'-O-methyltetrahydropapaveroline; 9, 6-O-methyltetrahydropapaveroline; 10, 2-O-methyltetrahydroxyberbine; 11, 11-O-methyltetrahydroxyberbine; 12, 10-O-methyltetrahydroxyberbine; 13, 3-O-methyltetrahydroxyberbine. (Fig. 6.1 and 6.2 were reproduced with permission from ref. 26, by courtesy of Marcel Dekker, Inc.).

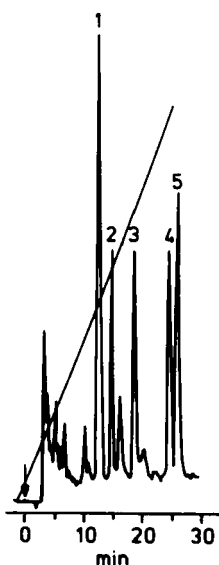


Fig. 6.3. HPLC analysis of curare sample⁹
Precolumn Corasil C18 (50x4.6 mm ID), column μ Bondapak C18 (300x4 mm ID), mobile phase A 0.025 M tetramethylammonium phosphate in methanol - water (1:3) (pH 4), B 0.025 M tetramethylammonium phosphate in methanol - water (9:11) (pH 4), linear gradient A+B (9:1) to (15:85) in 30 min, flow rate 1 ml/min, detection UV 280 nm. Peaks: 1, d-tubocurarine; 2, chondrocurine; 3, curarine; 4, isochondrodendrine; 5, curine.

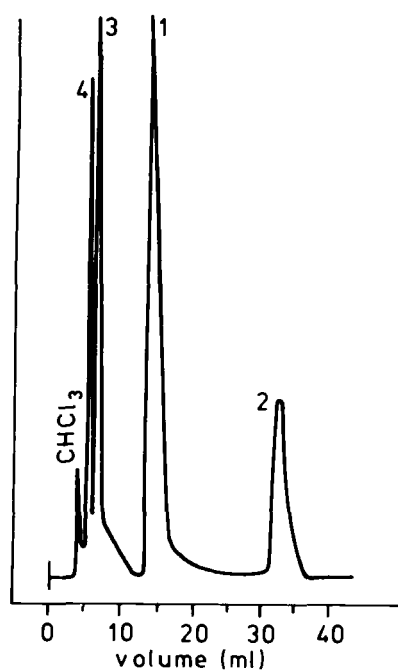


Fig. 6.4. Separation of some bisbenzylisoquinoline alkaloids¹⁵
Column μ Porasil (300x4 mm ID), mobile phase cyclohexane - chloroform - diethylamine (25:150:0.3), flow rate 2.3 ml/min, detection UV 254 nm. Peaks: 1, thalicarpine; 2, hernandalinol; 3, hernandaline; 4, dehydrothalicarpine.

TABLE 6.3

HPLC ANALYSIS OF VARIOUS ALKALOIDS INCLUDING ISOQUINOLINE ALKALOIDS

ALKALOIDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
Emetine,cephaeline, 22 other alkaloids	Analysis alkaloids	Merckosorb Si60, 5 μ m	300x2	CHCl ₃ -MeOH(9:1),(8:2),(7:3) Et ₂ O ₂ -MeOH(8:2),(7:3),(6:4)	2
Mescaline,opium alkaloids, quinine,cinchonine,strych- nine,nicotine,atropine,co- caine	Separation on ion-ex- change resins(ligand exchange LC)	Hydrolyzed Poragel T loaded with Cu ⁺⁺ Bio-Rad PC20,loaded with Cu ⁺⁺	470x6.3 470x6.3	0.06M NH ₄ OH in 33% EtOH 0.2 M NH ₄ OH in 33% EtOH 0.05M NH ₄ OH in 33% EtOH 0.03M NH ₄ OH in 33% EtOH	3
Emetine,Cinchona alkaloids, scopolamine,brucine,strych- nine,reserpine,yohimbine, caffeine	Detection with conduc- tance detector	Silica gel 10 μ m		CHCl ₃ -MeOH-hexane(7:3:10)	16
Dopamine,salsolinol,6-OMe-, 7-OMe,3'-OMe,4'-OMe-tetra- hydropapaveroline,2,3,9,10- tetrahydroxyberbine,2,3,10,- 11-tetrahydroxyberbine and its 11-OMe derivatives	Analysis dopamine derived isoquinoline alkaloids (Fig.6.1,6.2)	Partisil 10 SCX Vydac TP 401 SCX, 10 μ m Nucleosil SA, 10 μ m	250x4.6 250x3.2 250x3.2	0.1M NH ₄ H ₂ PO ₄ 0.2M NH ₄ H ₂ PO ₄ 0.5M NH ₄ H ₂ PO ₄ +5% n-BuOH	26
Emetine,cephaeline	Separation	Lichrosorb Si60, 5 μ m	200x2	CHCl ₃ -MeOH(85:15)	37

TABLE 6.4

HPLC ANALYSIS ISOQUINOLINE ALKALOIDS IN PLANT MATERIAL

ALKALOIDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
Dihydrochelerythrine, N-methylflindersine	Preparative HPLC plant extracts	Silica gel	300x50	Et ₂ O-hexane(1:9) EtOAc-hexane(1:4)	8
Tubocurarine,chondrocurine, curarine, isochondodendrine, curine	Analysis curare (Fig.6.3)	μ Bondapak C18	300x4	0.025M tetramethylammonium phosphate in A. MeOH-H ₂ O(1:3) pH 4, B. MeO-H ₂ O(9:11) pH 4, linear gradient A+B(9:1) to(3:17)	9

Berberine	Analysis crude drugs	Zipax SCX	1000x2.1	A. ACN-0.1M NaClO ₄ (3:2) B. 0.2M H ₃ BO ₃ -0.002M NaClO ₄ in H ₂ O(pH 8.5) A+B(7:3)	10,13
Berberine	Analysis in <i>Coptis</i> species	μBondapak C18	300x4	ACN-phosphate buffer (pH 5.2)(3:2)	11
Boldine	Analysis in Boldus extract	ODS-HC-Si1	250x2.6	MeOH-H ₂ O(1:19)	12
16 Cactus alkaloids (Table 6.2)	Analysis Cactus alkaloids	Lichrosorb Si60, 10 μm and Partisil 8 μm in series	300x4.5	ACN-conc. NH ₄ OH CHCl ₃ -1% conc. NH ₄ OH in MeOH(9:1)	14
Glaucine,dehydroglaucine, papaverine	Analysis in microbial cultures	μBondapak Phenyl	300x3.9	ACN-MeOH-0.05M KH ₂ PO ₄ (2:4:5)	24
Lycorine,ambelline,criglaucine	LC-MS of <i>Crinum</i> alkaloids	Spherisorb ODS, 5 μm	not given	ACN-H ₂ O-NH ₄ OH(79.3:20:0.3)	29
Berberine,palmitine	Histochemical chromatography	Lichrosorb RP8	not given	ACN-THF-0.1N tartaric acid-Na dodecyl-sulfate(20:20:59.5:0.5)	30
Ambelline	Detection Amaryllidaceae alkaloids with CD-detector	C8 bonded phase	150x5	MeOH-H ₂ O(3:2) containing a trace of NH ₄ OH	31
Emetine,cephaeline	Analysis Ipecacuanha	μPorasil	300x4.5	CHCl ₃ -(5% conc. NH ₄ OH in MeOH)(95:5)	38
Berberine,palmitine,cop-tisine	Analysis plant material	TSK gel LS-410, 5 μm	150x4	ACN-MeOH-0.1N tartaric acid-Na dodecyl-sulfate (40:10:49.5:0.5)	39
Emetine,cephaeline	Analysis Ipecacuanha	μPorasil	300x3.9	CHCl ₃ -MeOH-DEA(90:10:0.2)	40

TABLE 6.5

HPLC ANALYSIS ISOQUINOLINE ALKALOIDS IN PHARMACEUTICAL PREPARATIONS

ALKALOIDS	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
Emetine,cephaeline, codeine,morphine,nos- capine,ephedrine		Separation as dansyl deriva- tives(Fig.7.15)	Silica gel Si100	250x2.8	(isopr) ₂ O-isoprOH-conc.NH ₄ OH (48:2:0:3), (isopr) ₂ O sat. with conc.NH ₄ OH-isoprOH(99:1)	5,6,18
Emetine,atropine,di- hydroergotamine,bro- mocryptine,ephedrine	Pindolol,guan- facin,ketotifen, pizotifen,cle- mastine	Post-column derivatization with fluorescent pairing ion	Lichrosorb DIOL, 10μm Lichrosorb RP8, 10μm	250x4 100x4.6	0.1M phosphate buffer(pH 3) MeOH-0.02M phosphate buffer (pH 3)(3:2)	20

Emetine, cephaeline, opium alkaloids, various others	Sulfanilamide, phenytoine, phe- nobarbital	Identification pharmaceu- ticals (Fig. 7.14)	Partisil PXS 5/25	250x4.6	Et ₂ O sat. with 50-100% H ₂ O+ 0.65-0.8% DEA	22
Berberine	Acrinol	Analysis pharmaceuticals	Zorbax ODS	250	0.005M dodecylsulfate in ACN-H ₂ O (95:5)	28

TABLE 6.6

HPLC ANALYSIS ISOQUINOLINE ALKALOIDS IN BIOLOGICAL MATERIALS

ALKALOIDS	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. L x ID (mm)	MOBILE PHASE	REF
Thalicarpine, hernan- daline, hernandalinol, dehydrothalicarpine		Analysis in urine (Fig. 6.4)	μPorasil	300x4	Cyclohexane-CHCl ₃ -DEA (25:150:0.3)	15
Tubocurarine, isochon- dodendrocurine		Analysis in plasma	μBondapak C18	300x4	ACN-H ₂ O (18:82) containing 0.2 M HClO ₄ (pH 5.4)	17
Emetine	Naphtalene	Analysis in plasma	μBondapak C18	300x4	0.0025M octanesulfonate, 0.5% AcOH in MeOH-H ₂ O (56:44)	20
Mescaline	Various amines, amphetamines	Analysis stimulants in urine, precolumn derivatization	Lichrosorb Si100, 10 μm or Wakogel LC 5H, 5 μm	250x2	CHCl ₃ -EtOAc-EtOH-n-hexane (25:10:1:50)	25
N-n-propylnorapomor- phine, apomorphine, boldine		Analysis in plasma	μBondapak Phenyl	300x4	MeOH-ACN-acetate buffer (pH 3.5) (36:9:55) containing 0.001M Na-dodecylsulfate	27
Tubocurarine, alcu- ronium		Determination alcuronium in plasma	μBondapak C18	300x6.4	MeOH-H ₂ O (4:1) containing 0.25% AcOH and 0.005M Na-dodecylsul- fate	32
Tubocurarine		Determination in plasma	Radial-Pak C18, 10 μm	100x5	MeOH-(TrEA (10g/l)-pentanesul- fonic acid (1 ml)-H ₃ PO ₄ (2 ml)- H ₂ O ad 1l) (2:3)	33

Chapter 7

OPIUM ALKALOIDS

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HPLC has been used to try and solve a great number of problems concerning the analysis of opium alkaloids.

(1) The separation of the various components of naturally occurring mixtures of opium alkaloids.

(2) The separation of opium alkaloids and other compounds present in pharmaceutical preparations.

(3) The separation and identification of drugs of abuse.

In Tables 7.13-7.18 the different HPLC techniques that have been applied so far to solve the analytical problems mentioned, are summarized. They are arranged according to the aim of the analysis. A review of HPLC analysis of drugs abuse is given by Wheals³² and Gough and Baker¹⁰⁹.

7.1. ION-EXCHANGE HPLC

Depending on the analytical problem involved, a number of chromatographic systems have been used for the separation of opium alkaloids and related compounds. Twitchett and Moffat²¹ and Twitchett et al.^{27,30} evaluated several HPLC systems for the analysis of 30 drugs. Both microparticulate reversed-phase (octadecylsilane) and cation-exchange (sulfonic acid) columns were tested, the first in combination with methanol - aqueous phosphate buffer mixtures of varying pH, the latter with methanol - water (2:3) of varying pH and ionic strength. It was concluded that the reversed-phase columns had poor efficiency for most of the basic drugs tested^{21,30}, whereas the cation-exchange columns were found to be suitable for them^{27,30}; the optimum conditions could be selected by using eluents containing at least 40% methanol or acetonitrile, and by changing the ionic strength. Variation of the pH could influence the retention and the column efficiency; a pH of over 7 reduced the column life considerably.

The necessity of adding an organic solvent to the mobile phase for the analysis of alkaloids on ion-exchange columns has been outlined by Knox and Jurand^{4,5}. They investigated the separation of some opium alkaloids and analgesics by using strong anion or cation-exchange columns. Six major opium alkaloids were separated on a strong cation-exchange column by means of a boric acid buffer of pH 9.5, to which 4% acetonitrile and 1% *n*-propanol was added (Fig. 7.1). Twitchett¹⁸ applied the same method to the analysis of illicit heroin preparations (Fig. 7.2), whereas Wittwer⁶ used a gradient elution to separate the major opium alkaloids on a strong anion-exchange resin.

Walton and Murgat^{13,19,26} described the separation of some opium alkaloids and other compounds by means of ligand-exchange chromatography. Different cation-exchange resins loaded with metals that form ammonia complexes (Cu^{++} , Ni^{++} , Zn^{++} and Ag^{+}) were investi-

gated. As mobile phase ammonia-ethanol mixtures were used. Strong basic solvents may cause hydrolysis of some ester alkaloids, reducing the applicability of the method.

Hays et al.⁷ used Zipax SCX in purity analyses of heroin and morphine. The alkaloids were eluted with a gradient of sodium perchlorate (0.4-1.4 M) in a 0.01 M phosphate buffer of pH 6.8 containing 10% ethanol.

Matantseva and co-workers^{87,101,103} separated the major opium alkaloids on a pellicular sulfonic acid cation-exchange stationary phase, using a pH 4.5 phosphate buffer containing 30% acetonitrile as mobile phase.

7.2. REVERSED-PHASE HPLC

In one of the first HPLC separations described for the analysis of alkaloids^{2,3} a dynamic coating technique was used in order to apply the stationary phase (Poly G-300) on the silica gel support. Using various percentages of the stationary phase in the mobile phase, the loading of the support with the stationary phase could be varied. However, the availability of chemically bonded stationary phases has reduced the value of a dynamic coating procedure to merely a theoretically interesting one. Despite the fact that several authors to begin with reported poor results for the analysis of alkaloids with reversed-phase columns^{7,21,30,56}, an ever increasing number of HPLC separations with reversed-phase chromatography is being published. Honigberg et al.¹⁴ compared some chemically bonded stationary phases -on pellicular silica gel- in the analysis of cough-cold mixtures, containing, *i.e.*, some alkaloids. The authors found that the peak shape for a packing material with chemically bonded phenyl groups was more symmetrical than that with chemically bonded octadecyl groups, when equivalent mobile phases were used. Knox and Pryde²³ applied silica gel with chemically bonded short chain alkanes for the analysis of opium alkaloids. With 0.025 M ammonia in methanol - water (1:1), a good separation of the major alkaloids was obtained.

Various authors have discussed the factors influencing the retention behaviour of alkaloids, *i.e.*, opium alkaloids, in reversed-phase chromatography. Honigberg et al.¹⁴ found that for chemically bonded phenyl and octadecyl stationary phases a decrease of the acetonitrile content in a mobile phase consisting of acetonitrile and aqueous 0.1% ammonium acetate solution resulted in increased retention times and increasing band-spreading on both types of stationary phases. The retention times on octadecyl phases were usually longer than those on phenyl phases, when equivalent mobile phases were applied. Twitchett et al.^{21,27} reported that at pH 3 the retention time of morphine on an octadecyl phase column first decreased with an increase of the methanol content in the mobile phase and then increased. An increase of the pH led to an increase of the retention time of basic compounds (40% methanol as mobile phase). Thus, knowledge about the pK_a and the lipid solubility of a compound enables prediction about its retention behaviour on octadecyl columns.

According to Wu and Wittick³⁵ the retention of opium alkaloids on octadecyl columns is primarily governed by the percentage of acetonitrile in a mobile phase consisting of acetonitrile and an aqueous phosphate buffer. An increase of the acetonitrile content leads to a decrease of the retention times. The pH influences the retention: increase of the pH leads to an increased retention time. Although a baseline separation could be

TABLE 7.1

RETENTION VOLUME OF SOME OPIUM ALKALOIDS⁸⁰

Column, Hypersil ODS, 5 μ m (100x4.6 mm ID), mobile phase methanol - 0.01 M phosphate buffer (pH 3) containing 0.1 M potassium bromide (1:7), flow rate 0.75 ml/min.

Compound	Retention Volume (ml)	Compound	Retention Volume (ml)
Morphine-3-glucuronide	1.0	Dihydrocodeine	4.8
Normorphine	1.7	Nalorphine	4.9
Dihydromorphine	1.7	Codeine	5.3
Morphine	1.8	Norcodeine	5.7
Morphine-N-oxide	1.8	6-O-acetylmorphine	10.4
Codeine-N-oxide	4.3		

obtained with 0.1 M sodium dihydrogen phosphate in acetonitrile - water (1:3) (pH 4.8) (Fig. 7.3), the authors found that for quantitative analysis of morphine an acetonitrile concentration of 5% was more suitable. This HPLC system was also used for the determination of morphine in poppy straw⁵².

Poochikian and Cradock⁵⁷ used acetonitrile - aqueous phosphate buffers in combination with octadecyl columns for the analysis of heroin and its hydrolysis products. The retention times were found to be shorter with increasing percentages of acetonitrile and with more strongly buffered mobile phases. Also, the peak sharpness increased under such conditions. Optimum pH was between 5 and 8.7. The order of elution was not effected by changes in pH, buffer strength, or percentage of acetonitrile in the mobile phase. As well as mobile phases consisting of acetonitrile and aqueous buffers, phases in which acetonitrile has been replaced by methanol have been used in combination with octadecyl stationary phases (see Tables 7.13-7.18). The separation of codeine and morphine and some of their metabolites in such a system is summarized in Table 7.1⁸⁰.

TABLE 7.2

RETENTION TIMES OF SOME OPIUM ALKALOIDS⁷⁸

Systems A-D: column, 300x4 mm I.D. Nucleosil 10CN; systems E-G: column, 300x4 mm I.D. Nucleosil 10C18. Mobile phases: A, 1% ammonium acetate (pH 5.8)-acetonitrile-dioxane (80:10:10); B, 1% ammonium acetate (pH 5.8)-acetonitrile (80:20); C and E, 1% ammonium acetate (pH 5.8)-acetonitrile (70:30); D and G, 1% ammonium acetate (pH 5.8)-acetonitrile (60:40); F, 1% ammonium acetate (pH 5.8)-acetonitrile (65:35). All systems: flow-rate, 1.5 ml/min.

Alkaloid	Retention time (min)						
	A	B	C	D	E	F	G
Morphine	4.1	4.2	4.0	3.8	2.4	2.3	2.2
Codeine	5.1	5.4	4.8	4.5	3.3	3.0	2.9
Cryptopine	8.1	9.6	7.2	5.6	6.5	4.7	4.1
Thebaine	9.2	10.8	8.0	6.7	9.8	7.5	6.7
Noscapine	12.3	14.1	9.3	5.9	42.6	23.4	15.7
Papaverine	15.7	18.2	9.3	5.6	20.8	11.3	8.0

Wu and Dobberstein⁴¹ used methanol - 0.3% ammonium carbonate in water (4:1) as mobile phase in the quantitative analysis of thebaine in *Papaver bracteatum*. However, they reported that their octadecyl column had active sites on the silica gel support on which thebaine was adsorbed. For quantitative determinations it was necessary to saturate the active sites by injecting 20 µg thebaine once a day and then 5 µg thebaine several times - until successive injections gave thebaine peak areas which varied within 2% or less from each other.

Rasmussen et al.⁵¹ described a straight-phase and a reversed-phase HPLC separation for the determination of morphine in organic and aqueous poppy extracts respectively. The reversed-phase system consisted of an octadecyl column and methanol - 0.05 M aqueous ammonium carbonate (1:1) as mobile phase. This system gave better results for the analysis of morphine than the system mentioned by Wu and Wittick^{35,52} because of fewer interfering peaks. The separation was found to be greatly influenced by the salt concentration. The column performance was not affected by the high pH. The reversed-phase system had the advantage over the straight-phase system in that crude extracts could be analyzed. The straight-phase system could only be applied for more purified extracts.

Nobuhara et al.⁷⁸ separated opium alkaloids on octadecyl and cyanoalkyl phases using mixtures of acetonitrile and 1% aqueous ammonium acetate (pH 5.8) as mobile phase. Both types of column enabled baseline separation of the major opium alkaloids (Fig. 7.4 and 7.5, TABLE 7.2). However, the cyanoalkyl stationary phase gave the best separation for quantitative analysis.

Since no previously reported method adequately separated heroin from its major impurities and adulterants, Baker and Gough⁹³ tested various columns and mobile phases. Best results were obtained with an aminopropyl bonded phase (Fig. 7.6), the only disadvantage being the rather strong retention of morphine. For the analysis of alkaloids in *Papaver somniferum* plant material, an aminopropyl stationary phase has been used¹¹⁹, and except for noscapine and papaverine all major opium alkaloids were separated (Fig. 7.7).

For the analysis of acetylprocaine in heroin samples, Bernauer and Fuchs¹⁰⁵ employed an octadecyl stationary phase in combination with the mobile phase acetonitrile - water (91.5:8.5) containing 8 mg/100 ml tris(hydroxymethyl)-aminomethane (Fig. 7.8).

As noticed by other authors, Pettitt and Damon¹¹⁷ found that a phenyl-bonded phase gave better peak shapes for the alkaloids than an octyl or octadecyl type of stationary phase. However the addition of N,N-dimethyloctylamine to the mobile phase was found to be essential in order to greatly reduce tailing of opium alkaloids (Fig. 7.9).

Tatsuzawa et al.^{36,37,45,59} separated cold drugs and neuroleptics by using a styrene-divinylbenzene-methyl methacrylate copolymer as stationary phase. The best results were obtained with methanol - ammonia (99:1) as mobile phase. The effect of the pH and of the composition of the mobile phase on the separation were discussed. Aramaki et al.⁷⁰ analyzed a series of alkaloids on a macroporous styrene-divinylbenzene copolymer with alkaline acetonitrile - water mixtures as mobile phase (Fig. 7.10). The columns showed excellent stability, and also under the strong basic conditions used for the analysis of the alkaloids.

7.3. ION-PAIR HPLC

Because of the low effectiveness of reversed-phase columns for basic compounds - as reported by several authors - ion-pair chromatography has often preferred.

Lurie³⁸ analyzed drugs of forensic interest on octadecyl columns with 0.005 M 1-heptanesulfonic acid in methanol - water - acetic acid (40:59:1)(pH 3.5) (Table 7.3). The system enabled a simultaneous analysis of acidic, neutral and basic drugs. Among the compounds analyzed were the opium alkaloids (Fig. 7.11). A similar system was applied for the analysis of papaverine in plasma⁶⁷.

TABLE 7.3

REVERSED-PHASE ION-PAIR CHROMATOGRAPHY OF SOME DRUGS OF FORENSIC INTEREST³⁸

Column μ Bondapak C18 (300x4 mm I.D.), mobile phase 0.005 M heptanesulfonic acid in methanol - water - acetic acid (40:59:1), pH ca 3.5, flow rate 2 ml/min.

Retention volume (<i>R_r</i>) of compounds relative to noscapine			
Compound	<i>R_r</i>	Compound	<i>R_r</i>
Morphine	0.28	Thebaine	0.73
Codeine	0.36	Noscapine	1.00 (23.0 ml)
O-acetylmorphine	0.37	Quinidine	1.23
Procaine	0.38	Methapyrilene	1.24
Acetylprocaine	0.50	Papaverine	1.40
Acetylcodeine	0.69	Quinine	1.44
Heroin	0.70		

Olieman et al⁴⁰ found that reversed-phase ion-pair chromatography was the most suitable technique to analyze a series of morphinan derivatives. Normal reversed-phase chromatography on octadecyl columns, as well as straight-phase systems gave too much tailing - particularly for alkaloids with a high retention time (Table 7.4).

Smith et al.^{53,58,62} used ion-pair chromatography to separate apomorphine and related alkaloids. A diphenylsilane column was used in combination with methanol - acetonitrile - 0.02 M aqueous potassium dihydrogen phosphate - 0.03 M citric acid (pH 3.25) containing 0.001 M sodium dodecylsulfate (36:9:55). The system allowed baseline separation of apomorphine, apocodeine, *iso*apocodeine and the internal standard *N*-*n*-propylnorapomorphine or boldine. Dodecylsulfate as counter-ion gave better results than heptanesulfonic acid. Without the addition of a pairing-ion, tailing was observed.

Soni and Dugar⁶⁴ analyzed opiates with reversed-phase ion-pair chromatography; using the ion-pair chromatography no tailing for the alkaloids was observed on octadecylsilane columns. Tetrabutyl ammonium phosphate and 1-heptanesulfonic acid were used as pairing-ions (Table 7.5).

Kubiak and Munson⁸⁴ studied several pairing-ions for the analysis of morphine, codeine and ethylmorphine on octylsilane or octadecylsilane columns. Addition of 0.01 M ammonium nitrate to the mobile phase significantly improved the peak shape and reduced the tailing. In order to improve the separation further, different pairing-ions were tested in combination with a mobile phase consisting of 0.01 M ammonium nitrate in acetonitrile - water (375:625). Increase in the alkane chain length of the pairing-ion by up to eight carbons gave a relatively minor increase in the capacity factors of the alkaloids. However, further

TABLE 7.4

RETENTION TIMES (min) OF MORPHINANS FOR DIFFERENT SOLVENT SYSTEMS⁴⁰
(CONTAINING 0.005 M n-HEPTANESULFONIC ACID).

Column, μ Bondapak C18, (300x4 mm), flow rate 1.2 ml/min.

Dashes indicate no elution within a reasonable time (>30 min).

Compound	Solvent system		
	Methanol-water (50:50)	Methanol-water (40:60)	Acetonitrile-water (25:75)
Morphine	4.0	5.6	4.5
Codeine	4.6	7.5	6.1
Heroin	7.3	19.4	16.2
Dihydromorphine	4.1	6.0	4.9
Dihydrocodeine	4.5	7.7	5.7
Dihydrocodeinone	5.1	8.8	8.0
1-methyl-dihydrocodeine	5.4	9.6	7.6
1-methyl-dihydrocodeinone	5.9	12.7	11.5
1-bromo-dihydrocodeinone	8.0	18.7	17.6
1,7-dibromo-dihydrocodeinone	14.5	-	-
Dihydrothebaine	5.2	9.0	7.5
O-Acetyl-dihydrothebaine	5.5	10.1	8.7
N-nor-dihydrothebaine	5.1	9.5	6.7
N-formyl-dihydrothebaine	5.1,5.3	9.2,9.8	10.0
1-methyl-dihydrothebaine	6.0	12.6	10.1
N-formyl-1-methyl-dihydrothebaine	6.5,7.0	13.3,14.7	14.4
1-bromo-dihydrothebaine	8.0	21.4	14.0
1,7-dibromo-dihydrothebaine	11.7	-	-
2-hydroxy-dihydrothebaine	4.0	5.7	5.6
thebaine	7.5	17.9	17.5
oripavine	4.8	8.7	7.5

TABLE 7.5

REVERSED PHASE ION-PAIR CHROMATOGRAPHY OF SOME OPIUM ALKALOIDS AND THEIR ABSORPTION RATIOS RECORDED WITH DETECTION AT 254 AND 280 nm⁶⁴

Column μ Bondapak C18 (300x4 mm I.D.), mobile phase S₁ 0.01M tetrabutyl ammonium phosphate (pH 7.5) - methanol (53:47), S₂ 0.01M 1-heptane sulfonic acid (pH 3 with 1% acetic acid) - methanol (65:35) after 10 min changed to (45:55), flow rate 2 ml/min, detection UV 254 and 280 nm simultaneous.

Compound	Retention time (min)		Ratio 254/280	
	S ₁	S ₂	S ₁	S ₂
Acetylcodeine	2.5	...	5.25	...
Morphine	5.0	7.0	0.75	0.72
Oxymorphone	5.0	5.0	0.85	0.94
Noroxymorphone	5.2	3.8	0.87	0.84
Dihydromorphinone	5.8	...	0.77	...
Nalorphine	7.1	...	0.79	...
Codeine	8.3	12.5	1.10	1.05
O-acetylmorphine	10.1	9.0	0.59	0.54
Oxycodone	10.4	10.5	0.85	0.84
Dihydrocodeinone	11.8	retained	0.76	...
Heroin	15.4	...	0.24	...
Papaverine	16.7	...	10.1	9.9

increase in the chain length led to a considerable increase. Best results were obtained with dioctyl sodium sulfosuccinate. This pairing-ion circumvents the solubilization problem of some of the long chain alkanes pairing-ions (14 and 16 carbon atoms), but gives similar capacity factors as the long chain alkane pairing-ions mentioned. Morphine, codeine and ethylmorphine could be separated with 0.005 M dioctyl sodium sulfosuccinate in the mobile phase.

Eriksson et al.⁶³ used straight-phase ion-pair chromatography for the analysis of apomorphine in biological material. Best results were obtained with perchloric acid as pairing-ion. Dichloromethane - methanol - 1 M perchloric acid (956:40:4) was used as mobile phase in combination with a silica gel column.

Lurie and Demchuck^{98,99} and Lurie¹⁰⁰ extended their earlier studies on reversed-phase ion-pair HPLC of forensic drugs to the factors influencing the separation. The effect of the stationary phase, the water - methanol ratio in the mobile phase, and the alkyl-chain length of the counter-ion, as well as its concentration on the retention of a series of acidic, neutral and basic drugs, was studied. For solid supports containing chemically bonded octadecyl and phenyl stationary phases it was found that increased chain-length of the pairing ion (alkylsulfonic acid) led to an increase of the k' of basic compounds. This effect was less pronounced on a cyano-type stationary phase. A comparison of different stationary phases of cyano-, phenyl- and octadecyl types showed that the retention increased for basic compounds, from the cyano-type via the phenyl-type to the octadecyl-type, using the same mobile phase. However, the smaller the alkyl group of the counter-ion, the less pronounced was the effect. The concentration of the counter-ion had only a limited effect on the retention, whereas increased water content in the mobile phase led to longer retention times.

To improve the separation of certain compounds, the selectivity can be improved by increasing the percentage of water in the mobile phase by changing the counter-ion (different alkyl group) for solutes of different pK_a . Selectivity for basic compounds is little enhanced by different stationary phases, and the same holds true for alterations of the concentration of the counter-ion.

Two systems were developed that were suitable for the analysis of drugs of abuse¹⁰⁰. One consisted of a mobile phase of methanol - water - 1% acetic acid (40:59:1) containing 0.02 M methanesulfonic acid (pH 3.5) in combination with a microparticulate octadecyl column. It was recommended for samples containing compounds such as barbiturates, local anaesthetics, LSD and related alkaloids. The other system made use of the same column and a mobile phase of methanol - water - 1% acetic acid (20:79:1) containing 0.02 M methanesulfonic acid (pH 3.5). It was particularly suitable for the analysis of phenethylamines and for the separation of heroin and acetylcodeine. The increased counter-ion concentration in these mobile phases compared to previously used systems was applied to reduce the variation in retention times of bases for different samples. In a subsequent study¹²¹ Lurie et al. reported the analysis of illicit heroin samples using similar HPLC-systems; however, acetonitrile was used instead of methanol in order to allow faster flow rates, thus reducing analysis time. Instead of acetic acid the mobile phase was acidified with orthophosphoric acid to allow detection at 220 nm. The results are summarized in Table 7.6.

TABLE 7.6

RELATIVE RETENTION TIMES AND 220:254 ABSORBANCE RATIOS FOR HEROIN AND ITS ADULTERANTS AND BY-PRODUCTS¹.

S1: Column μ Bondapak C18 (300x3.9 mm I.D.), S2: column, Partisil 10 ODS-3 (250x4.6 mm I.D.) both with mobile phase 0.02 M methanesulfonic acid in acetonitrile - water - phosphoric acid (12:87:1)(pH=2.2 with 2 M sodium hydroxide), flow rate 3.0 ml/min.

Compound	relative retention times		220:254 ratio (S ₂)
	S ₁	S ₂	
L-Ascorbic acid	...	0.05	... ^a
Isonicotinamide	0.05	0.05	... ^a
Morphine	0.09	0.08	12.1
Aminopyrine	0.15	0.12	0.9
Procaine	0.12	0.12	4.1
Ephedrine	0.14	0.12	3.5
Paracetamol	0.11	0.12	0.6
Theophylline	0.12	0.13	1.4
Methapyrilene	0.15	0.13	2.0
Tripeleminamine	0.16	0.13	2.4
Codeine	0.17	0.16	8.9
Pyrimidine	0.18	0.17	3.2
Quinidine	0.19	0.19	0.7
Barbital	0.23	0.21	62.0
Quinine	...	0.22	0.7
O-Acetylmorphine	0.23	0.22	14.7
Caffeine	0.19	0.23	2.2
Phentermine	0.28	0.25	5.1
Lidocaine	0.30	0.30	16.6
Quinine (second peak)	...	0.30	0.7
Acetylprocaine	0.28	0.30	0.6
Prilocaine	...	0.31	3.5
Salicylamide	...	0.31	6.0
Antipyrine	0.36	0.36	1.4
Hyoscyamine	0.41	0.39	21.7
Strychnine	0.38	0.41	0.56
Benzocaine	0.50	0.58	4.9
Acetylsalicylic acid	0.60	0.58	10.7
Sodium salicylate	...	0.75	7.3
Tropacocaine	0.71	0.76	7.7
Phenobarbital	0.81	0.85	11.0
Benzoyltropeine	0.82	0.87	8.1
Acetylcodeine	0.85	0.88	11.2
Thebaine	0.88	0.92	4.6
Phenacetin	0.81	0.95	0.5
Meperidine	...	1.00	25.5
Heroin	1.00	1.00(19 min)	24.0
Cocaine	1.05	1.11	6.0
Amylocaine	...	1.37	5.9
Phencyclidine	2.12	2.15	8.5
Noscapine	2.30	2.36	10.8
Tetracaine	2.28	2.38	10.3
Papaverine	2.80	3.17	0.4
Tartaric acid
Diphenhydramine
Methadone
Phenylbutazone

^aElutes near solvent front.

^bExhibits no UV at 254 nm.

^cRetention time greater than 1 h.

Achhari and Jacobs⁸¹ (see CHAPTER 2) have made extensive studies of the ion-pair HPLC of basic drugs. Lindberg et al.⁹⁴ applied statistical optimization methods to the separation of some opium alkaloids, whereby four factors were varied: the eluent strength, the pH and the concentration of the phosphate buffer as well as the camphorsulfonic acid (the pairing-ion). Via a series of experiments the k' -value of each alkaloid as a function of the above mentioned variables was determined, and by statistical means the optimum conditions for a desired separation could be selected.

Reversed-phase ion-pair separations have been applied to the analysis of morphine and its metabolites in biological samples¹¹⁰ and to the analysis of the degradation products of heroin^{104,116,125} and morphine¹¹⁶.

7.4. STRAIGHT-PHASE HPLC

Opium alkaloids and drugs of abuse have been widely analyzed by straight-phase chromatography. Except for two investigations^{1,12} - in the early stage of development of HPLC - all HPLC analyses of opium alkaloids have been performed with silica gel columns in combination with basic solvents, in order to reduce tailing due to chemisorption of the alkaloid bases on the weakly acidic silica gel. Only once does aluminium oxide seem to have been used for the separation of drugs of abuse¹¹. In that case, a basic solvent system was used, *i.e.* 0.22% cyclohexylamine in cyclohexane or in cyclohexane - methanol (98.5:1.5).

Smith et al.^{8,10,17} analyzed the opium alkaloids by means of pellicular silica gel columns in combination with a mobile phase of *n*-hexane - chloroform - methanol - diethylamine in various ratios. Thebaine could be determined in *Papaver bracteatum* and *P. orientale* by means of an isocratic system⁸. By using a specially designed gradient system, the six major opium alkaloids could be determined in opium. Brucine was used as an internal standard^{10,17}.

Vincent and Engelke⁵⁴ carried out an isocratic HPLC separation of the five major opium alkaloids in *Papaver somniferum* and of thebaine in *P. bracteatum* on microparticulate silica gel for a quantitative determination of the alkaloids. A mobile phase consisting of four components: *n*-hexane - dichloromethane - ethanol - diethylamine (300:30:40:0.5) separated the five major opium alkaloids (TABLE 7.7, Fig. 7.12). By altering the ratio dichloromethane - ethanol, the retention of the alkaloids could be varied. Capsular tissue extracts could be analyzed directly.

Chan et al.¹¹ preferred cyclohexylamine as the basic modifier in the analysis of drugs of abuse on pellicular silica gel or aluminium oxide columns. Volatile amines were less suited, because the solvent composition changed with time, and secondary and tertiary amines absorbed UV-light at 254 nm.

Caude et al.^{25,48} used ethylamine as the basic modifier in a solvent system of ethyl acetate - methanol - water in order to analyze some opium alkaloids in pharmaceutical preparations on microparticulate silica gel columns, whereas Achhari and Theimer³⁹ used chloroform - methanol (3:1) containing 1% ammonia for the same kind of analyses - also on microparticulate silica gel. No drastic deterioration of the column efficiency was observed when ammonia was added to the solvent; the column was still usable even after two years.

TABLE 7.7.

RETENTION OF SOME PAPAVER ALKALOIDS AND RELATIVE DETECTOR RESPONSES^{1 54}Column, μ Porasil (300x4 mm I.D.), mobile phase *n*-hexane - dichloromethane - ethanol - diethylamine (300:20:20:0.5), flow rate 1.8 ml/min, detection UV 254 nm.

Alkaloid	R _t sec	R _x ²	Area ¹ (10 ³)	Alkaloid	R _t sec	R _x ²	Area ¹ (10 ³)
Morphine	1700	1.000	70	Cryptopine	193	0.114	360
Codeine	860	0.506	43	Alpinigenine	189	0.111	120
Salutaridine	780	0.459	500	Papaverine	175	0.103	16
Oripavine	680	0.400	11	Noscapine	170	0.100	150
Thebaine	374	0.220	118	Narceine	170	0.100	7
Laudanosine	370	0.218	60	Protopine	168	0.099	330
Isothebaine	330	0.194	270	Gnoscopine	160	0.094	130

¹Area in μ V/sec for each alkaloid at a concentration of 20 μ g/ μ l.²R_x=retention time relative to morphine

A similar solvent system was also used by Rasmussen et al.⁵¹ for the analysis of morphine in organic poppy extracts (Fig. 7.13). The extracts could be analyzed without any purification prior to HPLC analysis. Gimet and Filloux⁶⁰ performed analyses on alkaloids - including opium alkaloids - in pharmaceutical preparations, and used a microparticulate silica gel column and diethyl ether or diethyl ether saturated with water as mobile phase. In both cases 0.4% diethylamine was added to the mobile phase (Fig. 7.14). An increase of the percentage of saturation of the diethyl ether with water, as well as of the percentage of diethylamine led to a decrease of the retention times. Frei et al.²⁹ separated the dansyl derivatives of some alkaloids, *i.e.*, morphine, and some non-derivatized alkaloids (Fig. 7.15).

To circumvent the problems connected with the analysis of a wide range of drugs with often quite different polarities, Jane²² used microparticulate silica gel columns and very polar mobile phases, such as methanol - 2 M ammonia - 1 M ammonium nitrate (27:2:1) and methanol - 0.2 M ammonium nitrate (3:2) (Tables 7.8, and 7.9, Fig. 7.16). No noticeable loss of column performance was observed for routine use over several months with the HPLC system described. Baker et al.⁵⁶ used a similar system and found that the retention times of the drugs analyzed correlated quite well with those reported by Jane²² (Table 2.2,2.3).

The usefulness of such polar solvent systems in the analysis of opium alkaloids has also been demonstrated by Feher et al.⁶⁸ (Table 7.10).

Wittwer⁹¹ investigated the influence of the volatilization of amines in the mobile phase by testing the same solvent system, containing ammonia in various concentrations (without changing the water content of the mobile phase) in combination with a silica gel column. For the compounds tested, common adulterants or impurities of illicit heroin samples, only a few changes in the elution order were observed, particularly for the early eluting compounds, and furthermore an increase of retention time was observed upon decreasing ammonia concentration (Table 7.11). However, the relative retention varied little for most test compounds. The water content of the mobile phase was found to play an important role in the selectivity of the system. Retention times were reduced considerably on increase of the water content of the mobile phase but some compounds were more affected than others. Therefore, the water content of the mobile phase should be controlled

TABLE 7.8

RETENTION OF COMPOUNDS OF FORENSIC INTEREST RELATIVE TO MORPHINE²²

Column Partisil 6 μm (250x4.6 mm ID), mobile phase methanol - 2 M ammonia - 1 M ammonium nitrate (27:2:1), flow rate 1 ml/min, detection UV 278 nm (see also Fig.7.16).

Compound	R_{rel}	Compound	R_{rel}
Amethocaine	0.58	Methadone	0.74
Atropine	2.35	Methapyrilene	0.59
Antazoline	1.21	Methaqualone	0.45
Benzocaine	0.45	6-Methyldihydromorphine	1.26
Benztropine	2.88	6-O-acetylisopropylmorphine	0.66
Bromodiphenhydramine	0.65	6-O-acetylmorphine	0.75
Butacaine	0.58	Morphine	1.00(8.9 ml)
Caffeine	0.52	Nalorphine	0.55
Chlordiazepoxide	0.48	Nicotine	0.57
Chlorpheniramine	1.02	Nitrazepam	0.46
Chlorpromazine	0.67	Paracetamol	0.46
Cocaine	0.51	Meperidine	0.62
Diethazine	0.64	Phenacetin	0.45
Dextropropoxyphene	0.49	Phenbutrazate	0.45
Diazepam	0.45	Phencyclidine	0.66
Dihydromorphine	1.57	Procaine	0.56
Diphenhydramine	0.64	Quinidine	0.63
Oxymorphone	0.68	Quinine	0.65
Ethopropazine	0.61	Salicylamide	0.46
Heroin	0.69	Strychnine	1.57
Lignocaine	0.46	Theophylline	0.49
Meclophenoxate	0.57		

TABLE 7.9

RETENTION OF THE OPIUM ALKALOIDS RELATIVE TO MORPHINE²²

Conditions as in Table 7.8 (see also Fig.7.16)

Alkaloid	R_{rel}	Alkaloid	R_{rel}
Morphine	1.00(0.9ml)	Dihydrocodeinone	1.32
Codeine	0.95	Ethylmorphine	0.87
Thebaine	0.79	Dihydrocodeine	1.44
Papaverine	0.47	Oxycodone	0.60
Noscapine	0.47	Protopine	0.61
Narceine	0.92	Laudanosine	0.68
Cotarnine	4.10	Acetyldihydrocodeinone	0.74
Dihydromorphinone	1.50	(thebaine)	

TABLE 7.10

CAPACITY FACTORS OF SOME OPIUM ALKALOIDS IN STRAIGHT-PHASE AND REVERSED-PHASE SEPARATIONS⁶⁸

Straight phase HPLC-system: column, Partisil 5 μm (250x4.5 mm I.D.), mobile phase methanol - 2 M ammonia - 1 M ammonium nitrate (30:2:1), flow rate 0.9 ml/min.

Reversed phase HPLC-system: column, 10 μm Silica RP18 (250x4.0 mm I.D.), mobile phase, acetonitrile - 0.01 M ammonium carbonate (4:6), flow rate 2.5 ml/min.

Alkaloid	k' Straight-phase	k' Reversed-phase	Alkaloid	k' straight-phase	k' reversed-phase
Papaverine	0.0	5.07	Morphine	1.32	1.25
Noscapine	0.04	17.0	Dihydrocodeinone	2.20	7.02
Oxycodone	0.54	5.27	Dihydrocodeine	2.46	4.73
Thebaine	0.85	15.6	Dihydromorphinone	2.65	2.14
Ethylmorphine	1.12	4.64	Dihydromorphine	2.82	1.67
Codeine	1.21	2.97			

TABLE 7.11

EFFECT OF AMMONIA CONCENTRATION IN MOBILE PHASE ON THE RETENTION OF SOME COMPOUNDS RELATIVE TO HEROIN⁹¹

Column, μ Porasil (300x4 mm I.D.), mobile phase cyclohexane - (chloroform - methanol - ammonia(800:200:1))(3:1) S1: 28% ammonia; S2: 14% ammonia; S3: 7% ammonia, flow rate 2.0 ml/min, detection UV 254 nm.

relative retention vs. heroin in Solvent Systems							
Compound	S 1	S 2	S 3	Compound	S 1	S 2	S 3
Methaqualone	0.38	0.35	0.33	Phenobarbital	0.78	0.67	0.63
Diazepam	0.40	0.37	0.35	Tetracaine	0.79	0.76	0.77
Lidocaine	0.41	0.34	0.33	Acetylcodeine	0.82	0.81	0.81
Noscapine	0.44	0.39	0.36	Antipyrine	0.90	0.86	0.82
Cocaine	0.46	0.37	0.38	Heroin	1.00	1.00	1.00
Papaverine	0.48	0.44	0.41	Procaine	1.32	1.28	1.34
Aminopyrine	0.54	0.51	0.49	Acetylprocaine	1.56	1.54	1.60
Benzocaine	0.60	0.54	0.52	Codeine	1.98	1.90	1.94
Meperidine	0.63	0.62	0.62	6-O-acetylmorphine	2.06	2.05	2.11
Methapyrilene	0.64	0.64	0.66	Quinidine	2.21	2.39	2.44
Methadone	0.68	0.69	0.75	Quinine	2.48	2.66	2.67
Caffeine	0.72	0.68	0.66	Strychnine	2.86	3.00	3.03
Barbital	0.75	0.65	0.61	Morphine	5.72	5.35	5.56
Phenacetin	0.75	0.71	0.68	Retention time of heroin (sec)	323	353	377

thoroughly, and the water content of methanol particularly should be checked prior to its use.

Hansen⁹⁵ separated opium alkaloids on silica gel with both polar and non-polar solvents. He suggested an ion-exchange type of mechanism for the retention of the alkaloids in the polar solvent systems. The influence of the changes in the solvent composition on the k' of various alkaloids is shown in Fig. 7.17. The influence of pH and the polarity of the amine in the solvent system were tested. Increased retention was observed on increase of pH or increased polarity of the amines. The more polar amines gave rise to some peak asymmetry.

Umans et al.¹¹³ developed a separation on silica gel for the determination of heroin and its metabolites. The pH of 7 of the mobile phase: acetonitrile - methanol - (conc. ammonia - methanol (1:2)) - (acetic acid - methanol(1:1)) (75:25:0.04:0.216) avoided problems of column degradation and hydrolysis of heroin during the analysis and this mobile phase also allowed the more sensitive detection at 218 nm.

7.5. DETECTION

Detection of the opium alkaloids is usually performed at 254 nm and 280 nm. However, the intensity of the absorption varies for each alkaloid^{12,54} (Table 7.7). The ratio between the absorbance at 254 nm and 280 nm is a reproducible constant, characteristic for each compound⁵⁶. By using this absorbance ratio in combination with the relative retention times in three different HPLC systems, Baker et al. (Table 2.2, 2.3)⁵⁶ were able to identify 95% of a series of forensic drugs. The relative retention times and absorption ratios for 101 drugs were presented. A similar method was used for the identification of opiates by Soni and Dugar⁶⁴ (Table 7.5) and by Lurie et al.¹²¹; the latter authors, however, used

the ratio of absorbances at 220 and 254 nm (Table 7.6).

Although most investigators used 254 or 280 nm as the wavelength of detection, several preferred detection at ca. 220 nm^{92,96,113,121} or 235 nm¹⁰², because of the greater sensitivity.

Fell et al.¹⁰⁶ reported a rapid-scanning multichannel detection method and its application to the identification of heroin and related compounds. The detector was capable of simultaneously recording at three different wavelengths, while automatically capturing spectra on-line for storage and subsequent manipulation. The recorded absorption spectra could be transferred to their second or higher derivatives to improve the selectivity. Additional information could be obtained from absorbance ratios, calculated continuously throughout the chromatogram.

The natural fluorescence of the opium alkaloids has been used to obtain a selective and sensitive detection method. In this way codeine has been detected (the detector operating with an excitation wavelength of 213 nm and a cut-off filter for the emission of 320 nm)¹⁰⁸ and morphine (excitation 290 nm, emission 340 nm)⁹⁶.

Wheals²⁸, Ross⁴² and Frei⁵⁵ have reviewed the possibilities of improving the detection properties and selectivity by means of reaction chromatography. Jane and Taylor²⁰ used the oxidation of morphine to pseudomorphine for a specific and quantitative fluorimetric determination method for morphine in urine. Some morphine analogues also produced fluorescent dimers, but they could be separated during the HPLC. The oxidative dimerization was performed on the top of the HPLC column (silica gel) by injection of a mixture of the urine extract and 0.04 M potassium ferric cyanide. By introducing dihydromorphine as a reactive internal standard, the problem of variable reaction yields was overcome. Dihydromorphine undergoes the same oxidative dimerization as morphine, but a mixture of both alkaloids yields a mixed dimer. The three possible dimers were separated by HPLC and from the relative peak areas the morphine concentration could be determined. The retention time of a series of dimers is given in Table 7.12. For a large number of drugs it was reported that no interference was observed when this method was used. The absolute sensitivity determined by the detection limit of the fluorimeter was 4 ng.

TABLE 7.12

RETENTION TIMES OF THE PEAKS PRODUCED BY OXIDATION, RELATIVE TO THAT OF THE DIHYDRO-MORPHINE DIMER (6.1 min)²⁰

X	X-X	X-dihydro	Dihydro-dihydro	X	X-X	X-dihydro	Dihydro-dihydro
Morphine	0.47	0.66	1.00	Oxymorphone	retained		1.00
Normorphine	1.88	1.37	1.00	Dihydromorphinone	retained		1.00
Nalorphine	0.25	0.42	1.00	Pentazocine	0.38	0.57	1.00
6-O-acetylmorphine	0.31	0.50	1.00	Phenazocine	0.31	0.40	1.00
6-Methyldihydromorphine	three peaks which are not resolved			Paracetamol	0.32	0.45	1.00
Dihydrohydroxymorphine	0.54	0.71	1.00				

Column, Partisil 7 μ m, (250x4.6 mm I.D.), mobile phase methanol - 2 M ammonia - 1 M ammonium nitrate (3:2:1), flow rate 2 ml/min, detection, fluorimeter (excitation 320 nm, emission 436 nm).

Nelson et al.¹¹⁴ used the oxidation of morphine to pseudomorphine in a post-column reactor. For the post-column derivatizing reagent, a solution of 50 mg potassium ferric cyanide in 250 ml of 4 M ammonia was used. Methanol - 0.1 M aqueous potassium bromide (12.5:87.5) was used as mobile phase for the separation of the alkaloids on a octadecyl stationary phase. The fluorescence was measured at 432 nm after excitation at 323 nm. In addition to morphine, normorphine, dihydromorphine, nalorphine and 6-O-acetylmorphine gave fluorescent dimers. The results with the post-column reaction showed good agreement with those obtained with the method of Jane and Taylor²⁰.

Frei et al.²⁹ made the dansyl derivatives of some alkaloids in order to improve the detection limit and to save elaborated clean-up procedures in the analysis of pharmaceutical preparations. The dansyl derivatives were prepared by adding an excess of 0.1% dansylchloride in acetone and 0.1 M sodium carbonate to an aqueous solution of the alkaloid, heating at 45°C for 30 minutes and subsequently extracting the dansyl derivatives with benzene. The results of an analysis of a cough syrup containing codeine and noscapine is given in Fig. 7.15. In contrast to these alkaloids, morphine does react with dansylchloride to yield a mono-dansyl derivative.

Bollet et al.⁴⁸ described a new electrochemical detector for HPLC that was applied in the analysis of noscapine in pharmaceutical preparations using a straight-phase separation system. The sensitivity of the detection of noscapine was compatible with UV-detection, but impurities could only be detected by means of the electrochemical detector. White⁶¹ developed an electrochemical detector for the analysis of morphine in biological samples. Because of its phenolic group, morphine was most susceptible to electrochemical oxidation and thus gave the largest detector respons. Indoles and phenothiazines were oxidized less rapidly and consequently gave lower detector responses. Codeine gave no significant response at all. The method was quite specific for morphine, the sensitivity was less than 1 ng of morphine using an electrode potential of +0.60V (vs. silver/silver chloride reference electrode (SSCE)). Several authors reported the use of commercially available electrochemical detectors for the analysis of morphine and some morphine antagonists, electrode potentials of +1V⁶⁹, +0.8V⁷⁰, +0.725¹¹¹, +0.79¹¹² and +0.6-0.8V¹¹⁵ (all vs. SSCE) were employed. Codeine is not detected under such conditions. For the electrochemical detection of apomorphine an electrode potential of +0.5V (vs. SSCE) has been used⁸⁹.

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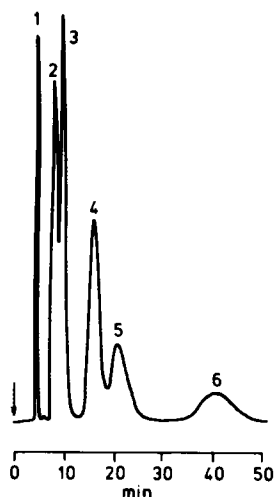


Fig. 7.1. Separation of some opium alkaloids⁴

Column Zipax SCX 37-44 μm (1200x2.1 mm ID), mobile phase 0.2 M sodium hydroxide, boric acid added to pH 9.5, 0.2 M KNO_3 with 4% acetonitrile and 1% *n*-propanol, linear velocity 0.4 cm/sec, detection UV 254 nm. Peaks: 1, morphine; 2, codeine; 3, papaverine; 4, thebaine; 5, cryptopine; 6, noscapine.

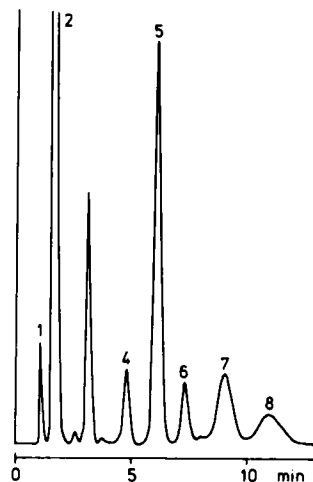


Fig. 7.2. Separation of some constituents of illicit heroin samples¹⁸

Column Zipax SCX 37-44 μm (1000x2.1 mm ID), mobile phase gradient elution with A: 0.2 M boric acid adjusted to pH 9.3 with 40% sodium hydroxide and B: 0.2 M boric acid - acetonitrile - *n*-propanol (86:12:2) adjusted to pH 9.8 with 40% sodium hydroxide, linear gradient from 0-100% B in 6 min, flow rate 2 ml/min, detection UV 270 nm. Peaks: 1, barbitone; 2, caffeine; 3, morphine; 4, O-acetylmorphine; 5, strychnine; 6, heroin; 7, quinine; 8, cocaine.

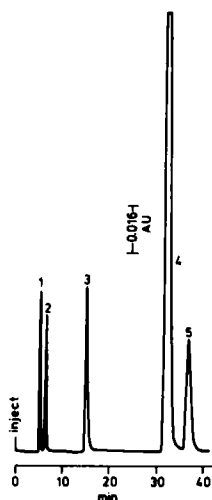


Fig. 7.3. Separation of major opium alkaloids³⁵

Column μ Bondapak C18 (300x4 mm ID), mobile phase 0.1 M sodium dihydrogen phosphate in acetonitrile - water(1:3). pH 4.8, flow rate 1.25 ml/min, detection UV 254 nm. Peaks: 1, morphine; 2, codeine; 3, thebaine; 4, papaverine; 5, noscapine. (Reproduced with permission from ref. 35, by courtesy of the American Chemical Society)

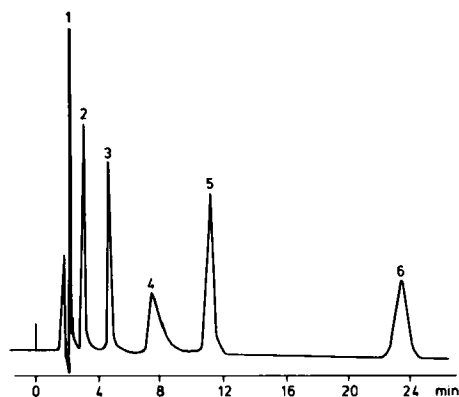


Fig. 7.4. Separation of some opium alkaloids.⁷⁸

Column Nucleosil 10C18 (300x4 mm ID), mobile phase 1% ammonium acetate (pH 5.8) - acetonitrile (65:35), flow rate 1.5 ml/min. Peaks: 1, morphine; 2, codeine; 3, cryptopine; 4, thebaine; 5, papaverine; 6, noscapine.

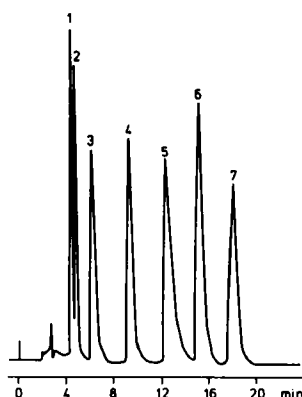


Fig. 7.5. Separation of some opium alkaloids.⁷⁸

Column Nucleosil 10CN (300x4 mm ID), mobile phase 1% ammonium acetate (pH 6.3) - acetonitrile - dioxane (79:16:5), flow rate 1.5 ml/min. Peaks: 1, narceine; 2, morphine; 3, codeine; 4, cryptopine; 5, thebaine; 6, noscapine; 7, papaverine.

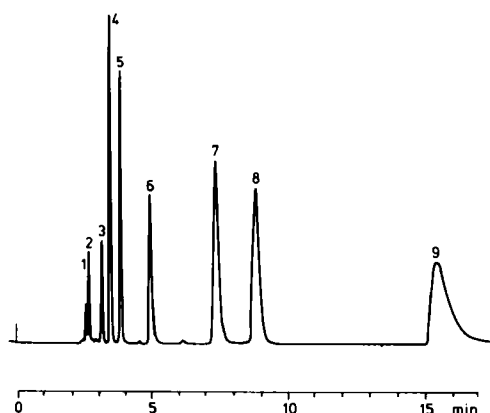


Fig. 7.6. Separation of heroin and some common adulterants and contaminations.⁹³

Column amino-propyl bonded silica (S5NH₂)(Phase-Sep) (250x4 mm ID), mobile phase acetonitrile - 0.005 M tetrabutylammonium phosphate (85:15), flow rate 1 ml/min, detection UV 284 nm. Peaks: 1, noscapine; 2, papaverine; 3, caffeine; 4, heroin; 5, acetylcodeine; 6, 6-O-acetylmorphine; 7, codeine; 8, strychnine; 9, morphine. (Reproduced with permission from ref. 93, by the courtesy of Journal Chromatographic Science)

Fig. 7.7. Separation of some opium alkaloids¹¹⁹
 Column Zorbax NH₂ (250x4.6 mm ID), mobile phase acetonitrile - 0.025 M potassium dihydrogen phosphate (3:1), flow rate 2.0 ml/min, detection UV 286 nm. Peaks: 1, papaverine; 2, thebaine; 3, narceine; 4, codeine; 5, morphine; 6, tyrosine (internal standard).

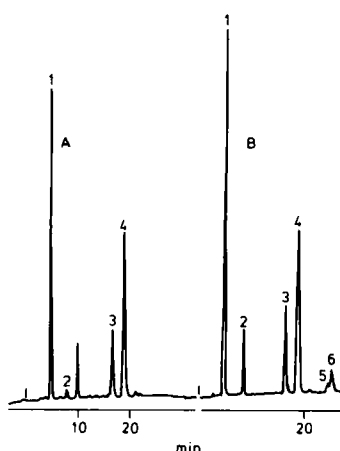
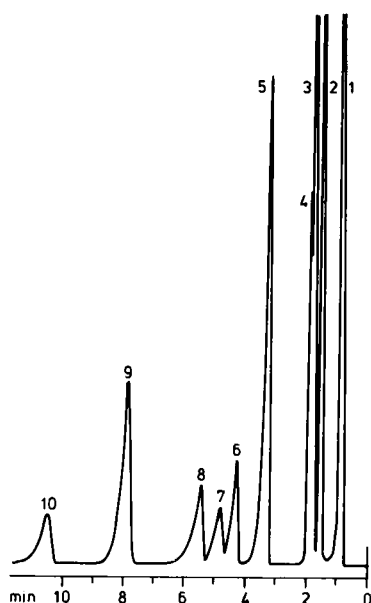
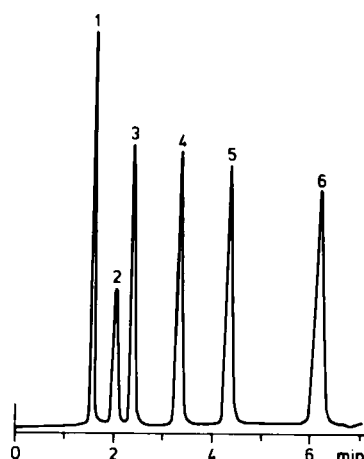


Fig. 7.8. Separation of heroin and some common adulterants and contaminations¹⁰⁵
 Column μ Bondapak C18 (300x4 mm ID), mobile phase acetonitrile - water (91.5:8.5) containing 8 mg tris(hydroxymethyl)aminomethane per 100 ml, flow rate 2 ml/min, detection UV 254 nm. Peaks: 1, ascorbic acid; 2, caffeine; 3, papaverine; 4, noscapine; 5, 6-O-acetylmorphine; 6, heroin; 7, morphine; 8, acetylcodeine; 9, acetylprocaine; 10 procaine.

Fig. 7.9. Separation of opium alkaloids¹¹⁷
 Column μ Bondapak Phenyl (250x5 mm ID), guard column Corasil C18 37-50 μ m (70x2 mm ID), mobile phase A: acetonitrile - water (5:95) and B: acetonitrile - water (20:80) both containing 1 ml/l N,N-dimethyloctylamine (pH 3.5 with sodium hydroxide), linear gradient from A to B in 20 min, flow rate 1.0 ml/min, detection UV 275 nm. Peaks: 1, morphine; 2, codeine; 3, thebaine; 4, quinine; 5, papaverine; 6, noscapine. Chromatogram A poppy-straw concentrate, B standard mixture.



Fig. 7.10. Separation alkaloids in opium extract⁷⁰
Column Hitachi gel 3010 10 μ m (220x4.6 mm ID), mobile phase acetonitrile - 0.019 M ammonia (48:52), flow rate 1 ml/min, temperature 65 $^{\circ}$ C, detection UV 254 nm. Peaks: 1, morphine; 2, codeine; 3, papaverine; 4, thebaine; 5, noscapine. (Reproduced with permission from ref. 70, by courtesy of the American Chemical Society).

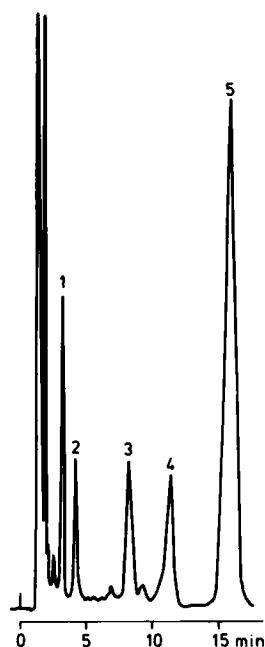


Fig. 7.11. Separation of some opium alkaloids³⁸
Column μ Bondapak C18 (300x4 mm ID), mobile phase 0.005 M heptanesulfonic acid in methanol - water - acetic acid (40:59:1) (pH ca. 3.5), flow rate 2 ml/min, detection UV 254 nm. Peaks: 1, morphine; 2, codeine; 3, thebaine; 4, noscapine; 5, papaverine. (Reproduced with permission from ref. 38, by courtesy of Journal Association official analytical chemists)

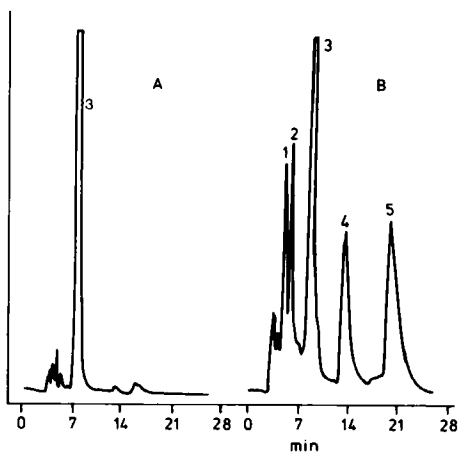


Fig. 7.12. Separation of major opium alkaloids⁵⁴
Column μ Porasil (300x4 mm ID), mobile phase n-hexane - dichloromethane - ethanol - diethylamine (300:30:40:0.5), flow rate 2.4 ml/min, detection UV 285 nm. Peaks: 1, noscapine; 2, papaverine; 3, thebaine; 4, codeine; 5, morphine. Chromatogram A *Papaver bracteatum* extract, B *Papaver somniferum* extract. (Reproduced with permission from ref. 54, by courtesy of Journal Association of official analytical chemists)

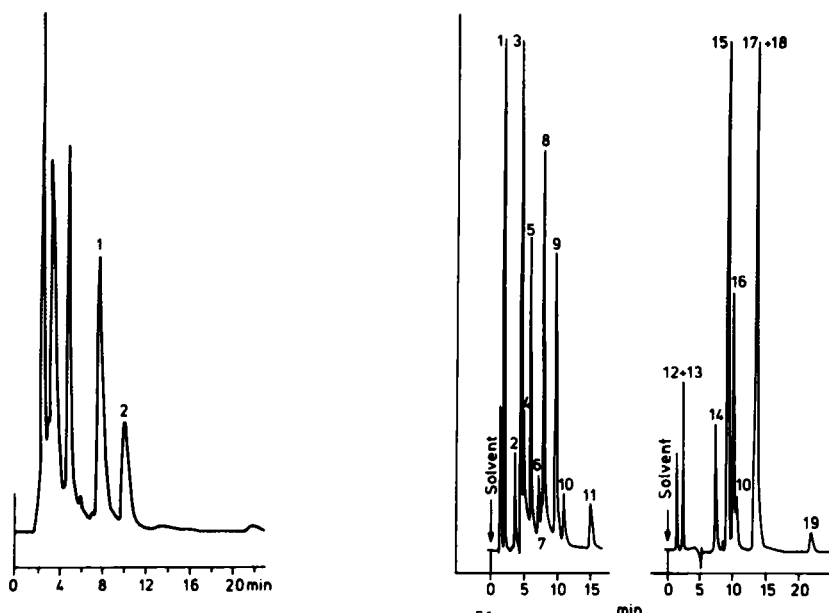


Fig. 7.13. Separation alkaloids from poppy extract⁵¹
 Column Spherisorb 5 μ m (250x3 mm ID), mobile phase methanol - chloroform - ammonia (18:81.5:0.5), flow rate 0.6 ml/min, detection UV 280 nm. Peaks: 1, morphine; 2, cinchonine (internal standard). (Reproduced with permission from ref. 51, by courtesy of Norsk Farmaceutisk Selskap).

Fig. 7.14. Separation of some alkaloids and drugs⁶⁰
 Column Partisil 5 μ m (250x4.6 mm ID), mobile phase diethyl ether 95% saturated with water + 0.4% diethylamine, flow rate 2 ml/min, detection UV 254 nm. Peaks: 1, noscapine; 2, aconitine; 3, papaverine; 4, emetine; 5, ephedrine; 6, cephaeline; 7, scopolamine; 8, ethylmorphine; 9, codeine; 10, phenytoine; 11 homatropine; 12, N-methylephedrine; 13, narceine; 14, quinine; 15, caffeine; 16, strychnine; 17, sulfanilamide; 18, atropine; 19, phenobarbital.

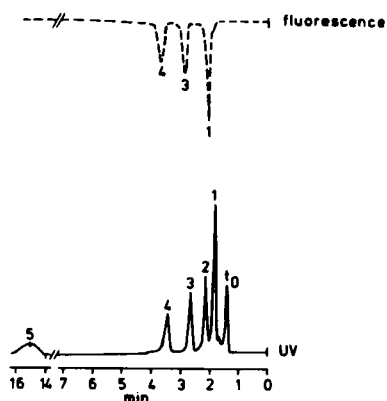


Fig. 7.15. Analysis of alkaloids in cough syrup after derivatization with dansyl-chloride²⁹
 Column Lichrosorb Si100 10 μ m (250x2.8 mm ID), mobile phase diisopropyl ether - isopropanol - conc. ammonia (48:2:0.3), detection UV 254 nm. Peaks: 1, Dns-ephedrine; 2, noscapine; 3, Dns-cephaeline; 4, Dns-emetine; 5, codeine; t_R, dodecylbenzene. Fluorescence detection with excitation 360 nm and emission 500-510 nm.

TABLE 7.13

HPLC ANALYSIS OF VARIOUS COMPOUNDS INCLUDING OPIUM ALKALOIDS

ALKALOIDS*	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID (mm)	MOBILE PHASE	REF.
C,M,Na,No,P,T, 18 others		Analysis alkaloids (Table 2.4)	Merckosorb Si60 5 μ m	300x2	CHCl ₃ -MeOH(9:1),(8:2),(7:3) Et ₂ O-MeOH(8:2),(7:3),(6:4)	12
C,M,No,P,EtM,Meth, coc,A,S,nic		Separation on ion-exchange resins (ligand-exchange LC)	Hydrolyzed Poragel PT loaded with Cu ²⁺ or Ni ²⁺	470x6.3	0.06M NH ₄ OH in 33% EtOH	13
C,M,No,P,EtM,Meth,Q, cinchonine,coc,A,S,nic		Separation on ion-exchange resins (ligand-exchange LC)	Hydrolyzed Poragel PT loaded with Cu ²⁺ Bio-Rad, PC20 loaded with Cu ²⁺	470x6.3 470x6.3	0.06M NH ₄ OH in 33% EtOH 0.2 M NH ₄ OH in 33% EtOH 0.05M NH ₄ OH in 33% EtOH 0.03M NH ₄ OH in 33% EtOH	19,26
C,Na,B,aconitine,caf, colchicine,cinchonidine	Santonine	Effect solvent composition on retention	Lichrosorb RP2 10 μ m	120x3.5	MeOH-H ₂ O (1:4),(2:3),(3:2),(4:1) MeOH	66
C,P,A,scop,Qd,caf	Various drugs	Retention behaviour basic drugs in ion-pair HPLC	μ Bondapak C18 μ Bondapak Phenyl μ Bondapak CN μ Bondagel Chromegabond C8 Chromegabond C6H11	300x4	0.005M heptanesulfonic acid in MeOH-H ₂ O-AcOH(49:50:1)(pH 4.0)	81
C,M,diHC,Mep,prop,A, ajmaline,caf,Tp,Q,Qd	Various drugs	Separation basic drugs with non-aqueous ionic solvents on silica gel	Syloid 74 silica Spherisorb S5W silica	250x4.9 250x4.9	MeOH or MeOH-CHCl ₃ (2:3) containing 1.42 ml HClO ₄ and 3.68 ml conc. NH ₄ OH per l (pH 9.2) MeOH-hexane(85:15) containing 0.02, 0.05 or 0.10% HClO ₄	118

TABLE 7.14

HPLC ANALYSIS IN THE PURITY CONTROL OF OPIUM ALKALOIDS

ALKALOIDS*	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
C,M,H,AcC,30AcM,60AcM	Purity profiles M, H, MHC1	Zipax ETH Zipax ODS Zipax SCX	1000x2.2 1000x2.2 1000x2.2	MeOH-H ₂ O(4:6) pH 8.6 ⁻ phosphate buffer 0.4-1.4M NaClO ₄ in 0.01M pH 6.8 phosphate buffer containig 10% EtOH,gradient elution	7

* For abbreviations see footnote Table 7.18

C,M,Eph	Stability control	μ Bondapak C18	300x4	0.1M KH_2PO_4 -MeOH(9:1) 0.1M KH_2PO_4 -EtOH(52.5:47.5)	31
M,H,30AcM,60AcM	Study of the hydrolysis of H	μ Bondapak C18	300x3.9	ACN-0.015M KH_2PO_4 (pH 3.5)(3:7)	57
M,P,H,0AcM	Stability H in CHCl_3 - H_2O	μ Bondapak C18	300x4	ACN-phosphate buffer (pH 3.5) (85:15)	102
M,H,60AcM	Stability H in CHCl_3 - H_2O at various pH	Hypersil ODS 10 μm	100	0.01M Na-pentanesulfonate-ACN- H_3PO_4 (69.5:30.0:0.5)(pH 2.0)	104,125

TABLE 7.15

HPLC ANALYSIS OPIUM ALKALOIDS IN PLANT MATERIAL AND OPIUM

ALKALOIDS*	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
C,M,No,P,T,crypt	Separation by means of ion-exchange LC (Fig.7.1)	Zipax SCX 37-44 μm	1000x2.1	(0.2M NaOH, boric acid added to pH 9.5, 0.2M KNO_3)-ACN-prOH(95:4:1)	4
C,M,No,P,T,Losine,crypt	Determination M in opium	Zipax SAX	1000x2.1	A. 0.01M boric acid buffer pH 9.5 with 1M NaOH B. 0.01M KH_2PO_4 buffer pH 6.0 with 1 M NaOH gradient A+B(85:15) to B (5% or 10% per min, linear)	6
T,isoT,orientalidine	Determination T in poppy plants	Corasil II 37-50 μm	300x2.8	n-Hexane- CHCl_3 -MeOH-DEA(900:75:25:0.1)	8
C,M,No,P,T,crypt	Separation opium alkaloids	Corasil II 37-50 μm	500x2.8	A. CHCl_3 -MeOH-DEA(100:300:1) B. Hexane special gradient system start with 30 ml A in 3 l B	10
C,M,No,P,T,crypt,B	Simultaneous assay alkaloids in opium	Corasil II 37-50 μm	1000x2.8	as in 10, gradient starts with 15 ml A in 1 l B	17
C,M,Na,No,P,T,cot,Losine,prot	Separation alkaloids in opium (Table 7.8,7.9 and Fig.7.16)	Partisil 6 μm	250x4.6	MeOH-2M NH_4OH -1M NH_4NO_3 (27:2:1)	22
C,M,No,P,T,crypt	Performance of new short chain reversed phase packing	Silica gel SAS 6 μm	125x5	0.025M NH_4OH in MeOH- H_2O (1:1)	23
C,M,No,P,T	Separation alkaloids from opium	Partisil 5	250x4.9	MeOH-2M NH_4OH -1M NH_4NO_3 (27:2:1)	32
C,M,No,P,T	Determination C, M and T in opium (Fig.7.3)	μ Bondapak C18	300x4.0	0.1M NaH_2PO_4 in ACN- H_2O (5:95) 0.1M NaH_2PO_4 in ACN- H_2O (25:75) pH adjusted to 2.0, 4.8 or 7.1	35

C,M,No,P,T	Separation alkaloids from opium (Table 7.3 and Fig.7.11)	μBondapak C18	300x4	0.005M Heptanesulfonic acid in MeOH-H ₂ O-AcOH(40:59:1)(pH 3.5)	38
C,M,T,isoT,orientalidine	Determination T in <i>Papaver bracteatum</i>	μBondapak C18	300x4	MeOH-0.3% aq. (NH ₄) ₂ CO ₃ (4:1)	41
C,No,P,T	Separation	Polystyrene beads	no details	MeOH-n-hexane-28% NH ₄ OH(97:2:1)	46
M,cinchonine	Determination M in aqueous and organic poppy extracts (Fig.7.13)	Spherisorb ODS 10 μm Spherisorb silica 5 μm	250x3 250x3	MeOH-0.05M aq.(NH ₄) ₂ CO ₃ (1:1) CHCl ₃ -MeOH-NH ₄ OH(81:5:18:0.5)	51
M	Determination M in poppy straw	μBondapak C18	2 columns 300x4	0.1M NaH ₂ PO ₄ in ACN-H ₂ O(6:94)	52
C,M,Na,No,P,T,isoT,prot,rypt, Losine,oripavine,salutaridine, alpinigenine,gnoscopine	Determination of alkaloids in <i>Papaver bracteatum</i> and <i>P.somniferum</i> (Table 7.7, Fig.7.12)	μPorasil 5 μm	300x4	n-Hexane-CH ₂ Cl ₂ -EtOH-DEA(300:30:40:0.5)	54
C,M,No,P,T	Analysis alkaloids in opium (Table 7.10)	Partisil 7 μm Silica RP18 10 μm	250x4.5 250x4.0	MeOH-2M NH ₄ OH-1M NH ₄ NO ₃ (30:2:1) ACN-0.005M ⁴ aq.(NH ₄) ₂ CO ₃ (4:6)	68
C,M,No,P,T,EtM,A,Eph,RSP,S,C,Q, yohimbine,acridine	Separation alkaloids on styrene-divinyl-benzene polymer (Figs. 7.10 and 8.6)	Hitachi gel 3010 10 μm	220x4.6	ACN-0.02M NH ₄ OH(3:2) ACN-0.02M tetrabutylammonium hydroxide (3:7),(2:3)	70
C,M,T,norM	Analysis M in opium	Lichrosorb Si60 5 μm	150x4.6	1,2-Dichloroethane-MeOH-AcOH-DEA-H ₂ O(80:20:1:0.5:1)	71
C,M,Na,No,P,T,rypt	Determination alkaloids in opium (Table 7.2, Figs.7.4 and 7.5)	Nucleosil 10CN	300x4	1% aq. NH ₄ OAc(pH 6.3)-ACN-dioxane (79:16:5) ⁴ 1% aq. NH ₄ OAc(pH 5.8)-ACN-dioxane (8:1:1) 1% aq. NH ₄ OAc(pH 5.8)-ACN(4:1), (3:2),(7:3)	78
		Nucleosil 10C18	300x4	1% aq. NH ₄ OAc(pH 5.8)-ACN(7:3), (3:2),(65:35)	78
C,M,No,P,T	Determination in raw materials	Bondapax CX/Corasil	1000x2.1	ACN-phosphate buffer(pH 4.5)(3:7)	103
C,M,No,P,T,Q	Analysis of poppy straw concentrate (Fig.7.9)	μBondapak Phenyl	250x5	A. ACN-H ₂ O(5:95) B. ACN-H ₂ O(20:80) both containing 1ml/l AcOH and 0.04 ml/l N,N-dimethyloctylamine Linear gradient: A to B(20 min)	117
C,M,Na,No,P,T	Analysis <i>Papaver somniferum</i> plant material and latex(Fig.7.7)	Zorbax NH ₂	250x4.6	ACN-0.025M KH ₂ PO ₄ (3:1)	119

*For abbreviations see footnote Table 7.18

TABLE 7.16

HPLC ANALYSIS OPIUM ALKALOIDS IN PHARMACEUTICAL PREPARATIONS

ALKALOIDS*	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
OxyC,caf,omatropine	Acsal,phen, hexobarb	Quantitative analysis multi- component drugs	Zipax WAX	1000x2.1	1.5M Na ₂ SO ₄ 0.005M HNO ₃	9
C,diHCone,Eph,S, methylomatropine	Various anti- tussives,anti- histaminics and analgesics	Analysis of cough-cold mix- tures	Corasil C18 or Corasil Phenyl	1220x2.3	ACN-0.1% aq.(NH ₄) ₂ CO ₃ (pH 8.5)(1:1) ACN-0.1% aq.(NH ₄) ₂ CO ₃ (pH 8.6)(3:2) ACN-0.1% aq.(NH ₄) ₂ CO ₃ (pH 8.9)(9:1) ACN-1% aq.(NH ₄)OAc (pH 7.04)(1:4) ACN-1% aq.(NH ₄)OAc (pH 7.4)(3:2) ACN-1% aq.(NH ₄)OAc (pH 7.58)(4:1)	14
No,P,Tp	Promethazine, phenobarbital	Analysis pharmaceuticals	Spherosil 5 µm	100x4.8 150x4.8	EtOAc-MeOH(9:1) EtOAc-MeOH-50% aq. ethylamine (97:2.94:0.06)	25
C,M,No,cephaeline, emetine,Eph		Separation as dansyl deriva- tives (Fig.7.15)	Silica gel Si100	250x2.8	(Isopr) ₂ O-isoprOH-conc.NH ₄ OH(48:2:0.3) (Isopr) ₂ O sat. with conc. ⁴ NH ₄ OH- isoprOH(99:1)	29
M		Determination M in paregoric USP	µBondapak C18	300x4	0.1M KH ₂ PO ₄ buffer in H ₂ O-MeOH (93:7)	33
No,Dmethphan,caf	Antitussives, expectorants, antihistaminics	Analysis anticold drugs	Porous styrene-divinyl benzene polymer or polymethacrylate gel	no details	MeOH-NH ₄ OH	36,37
C,P,Dmethphan,atro- pine,omatropine,S, Q,Qd,HQd,scopo,eph, xanthines	Various drugs	Analysis pharmaceuticals	Partisil 10 µm	250x4.6	CH ₂ Cl ₂ -MeOH(1:3) with 1% conc.NH ₄ OH	39
C,M,T,H,oripavine, 16 synthetic deri- vatives		Separation (Table 7.4)	µBondapak C18	300x4	MeOH-H ₂ O(1:1),(2:3) containing 0.005M ⁺ heptanesulfonic acid idem in ACN-H ₂ O(1:3)	40
C	Various drugs	Analysis expectorants	µBondapak C18	300x4	0.05M KH ₂ PO ₄ in H ₂ O-MeOH(87:13)	43
No,Dmethphan	Antitussives, expectorants, antihistaminics	Analysis pharmaceuticals	Polymethacrylate	no details	MeOH-28% NH ₄ OH(99:1)	45
No	Analgesics	Analysis with electrochemi- cal detector	Partisil 5 µm	150x4.8	EtOAc-MeOH-H ₂ O-ethylamine (782:200:6:2)	48

M	Methylparaben propylparaben	Analysis M in injectables	μBondapak C18	300x4	A. MeOH B. 0.1% aq. NaH ₂ PO ₄ solution containing 5% MeOH (pH 4.0), A+B(3:2)	50
No, caf, Dmethphan	Antitussives, expectorants, antihistaminics various others	Separation on porous polymer resins	Styrene-divinylbenzene methyl methacrylate co- polymer, substituted with hydroxymethyl groups	500x5 or 500x3	MeOH-NH ₄ OH(99:1) MeOH-H ₂ O-NH ₄ OH(95:5:1)	59
C, Na, No, P, EtM, Q, S, caf, A, Eph, aconitine, scopo, homatropine, emetine, cephaeline		Identification in pharmaceuticals (Fig. 7.14)	Partisil PXS 5/25	250x4.6	Et ₂ O sat. with 50-100% H ₂ O + 0.05-0.8% DEA	60
C, M, No, P, T, EtM, oxyC, diHC, diHCone, diHM, diHMone, caf		Analysis pharmaceuticals	Partisil 7 μm Silica RP18 10 μm	250x4.4 250x4	MeOH-2M NH ₄ OH-1M NH ₄ NO ₃ (30:2:1) ACN-0.005M aq. (NH ₄) ₂ CO ₃ (4:6)	68
C, caf	Acsal, Salam, phen, par	Analysis analgesics	μBondapak C18	300x4	0.01M KH ₂ PO ₄ buffer in H ₂ O-MeOH (81:19) pH 2.3 or 4.85	83
C, M, EtM		Analysis syrups	μBondapak C18 or RP10A	300x4 250x4.6	0.01M NH ₄ NO ₃ in ACN-H ₂ O(375:625) containing 0.005M dioctylsulfosuccinate, pH 3.3 with AcOH	84
M, H, 60AcM, coc, benzoyllecgonine	Benzoic acid	Stability H and coc in pharmaceutical dosage form	μBondapak C18	300x4	ACN-0.015M Na ₂ HPO ₄ (pH 3.0)(1:3)	85
C, M, apoM, nalo, nalox	Par, 4-amino- phenol	Analysis pharmaceuticals	Lichrosorb Si60 10 μm	250x2.1	CH ₂ Cl ₂ -MeOH-n-hexane-NH ₄ OH(232:44:723:1)	86
M, pseudoM, M N-ox, coc		Analysis in injections	Partisil SCX	250x4.6	0.7% KH ₂ PO ₄ in MeOH-H ₂ O(1:3)(pH 2.0) A. 0.15% KH ₂ PO ₄ in MeOH-H ₂ O(1:19) B. 1.5% KH ₂ PO ₄ in MeOH-H ₂ O(2:3) linear gradient A to B (10% B/min)	88
ApoM, N-n-propylnor- apoM		Determination in tablets	μBondapak Phenyl	300x4	THF-0.05M KH ₂ PO ₄ , 0.001M methane-sulfonate(15:85) MeOH-ACN-0.05M KH ₂ PO ₄ (5:15:80)	89
C, M	Chlorocresol, methyl 4-hydroxybenzoate	Determination M in injections	Lichrosorb RP18 10 μm	100x4.6	ACN-0.75% aq. NH ₄ OAc(pH 7.0)(3:7), (1:4)	90
C, M, No, P, T, methyl- homatropine		Determination in tablets and injections	Nucleosil 5C8	120x4.6 (2x)	ACN-0.01M aq. phosphate buffer (pH 5.0)(2:3)	92
C, M, No		Determination in pharmaceuticals	Bondapak CX/Corasil	500x2.1	ACN-0.1M KH ₂ PO ₄ (pH 4.8)	101
M, H, 60AcM, M N-ox, pseudoM		Determination degradation products M and H	Hypersil ODS 5 μm Ultrasphere ODS 5 μm	100x5 250x4.6	0.01M aq. pentanesulfonate-ACN-H ₃ PO ₄ (69.5:30:0.5)(pH 2.0) idem (69.95:30:0.05)(pH 2.6)	116

*For abbreviations see footnote Table 7.18

C,Eph,Dmethphan	Antitussives, antihistami- nics	Determination in cough-cold preparations	μ Bondapak C18	300x4	5.8g Na dioctylsulfosuccinate in MeOH-H ₂ O-THF-85% H ₃ PO ₄ (680:290: 40:1) (pH 3.8)	122
C,diHCone,diHMone	Par	Determination in tablets	μ Bondapak C18	300x4	MeOH-0.01N KH ₂ PO ₄ , 0.05M KNO ₃ buffer pH 4.5(1:3)	123
C	Par,acsal	Determination in tablets and suppositories	Nucleosil 10C18	300x4	MeOH-H ₂ O(9:16) containing Na octyl- sulfonate	124

TABLE 7.17

HPLC ANALYSIS OPIUM ALKALOIDS IN BIOLOGICAL MATERIAL

ALKALOIDS	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
C,M,coc,caf,Tp		Detection in urine	BOP(no further details)		Heptane-prOH(9:1)	16
M,diHM		Detection in urine(Table 7.12)	Partisil 7 μ m	250x4.6	MeOH-2M NH ₄ OH-1M NH ₄ NO ₃ (3:2:1)	20,28
Opiates	Opioid pepti- des	Ion-pair chromatography with μ Bondapak C18 TFA		300x4	5mM TFA(pH 2.5) in linear gradient of 30-50% ACN(10 min, 2ml/min) 10mM HCOOH-MeOH(1:1) 5mM TFA-MeOH(1:1)	49
ApoM,N-n-propylnor- apoM,apoC,isoapoC, boldine		Determination in serum	μ Bondapak Phenyl	300x4	MeOH-ACN-buffer(0.02M KH ₂ PO ₄ -0.03M citric acid, pH 3.25, containing 0.001M dodecylsulfate)(36:9:55)	53,58,62
C,H,M,oxym,60AcM, diHM,diHMone,MediHM, Nalox,keto,lev,norlev, dextrorphan,cephaeline, psilocin,caf,LSD	Various drugs	Electrochemical detection, analysis M in blood	Silica Syloid 74 7 μ m	200x4.6	MeOH-NH ₄ NO ₃ buffer pH 10.2(9:1)	61
ApoM		Determination in plasma or tissues	Lichrosorb Si100 10 μ m	200x3	CH ₂ Cl ₂ -MeOH-1M HClO ₄ (956:40:4)	63
C,M,Mep	Barbiturates	XAD ₂ extraction method	XAD ₂		Various solvents	65
P	Chlorphenira- mine	Determination in plasma	Partisil ODS 10 μ m		MeOH-1% aq. AcOH and 0.005M hep- tanesulfonic acid (55:45)	67
C,M,nalo,Meth	Par,benzodia- zepines	Determination M in serum, electrochemical detection	Lichrosorb RP18	300x4	MeOH-0.01M KH ₂ PO ₄ (85:15)	69
C,M,diHCone		Determination in urine	Spherisorb ODS 5 μ m	250x4	0.1M NaH ₂ PO ₄ in ACN-H ₂ O(1:3)	72
P,ethavarine		Determination in plasma	Partisil ODS 10 μ m	250x4.6	MeOH-0.1% aq.KH ₂ PO ₄ (65:35)	75

P, Losine, caf, Tb, Tp	Chlorthiazides tetracyclines	Determination in urine and plasma	C8 reversed-phase 10 μ m	250x4.6	MeOH-0.015M Na-borate buffer pH 8.5 (58:42)	76
C, M, nalo, oxyM, nalo, pentazocine, naltrexone		Amperometric detection, de- termination in plasma	μ Bondapak C18	300x4	MeOH-H ₂ O(1:4) containing 0.05M Bu ₄ N, pH 6.3, adjusted with H ₃ PO ₄	77
C, norC, C N-ox, M, norM, M N-ox, 60AcC, diHC, diHM, M-3-glucuronide, C-6-glucuronide, nalo		Combined HPLC-immunoassay method for analysis M, C and metabolites in biological fluids (Table 7.1)	Hypersil ODS 5 μ m	100x4.6	MeOH-0.01M phosphate buffer, 0.1M KBr(pH 3)(1:7)	80
C, M, Mep, caf	Amphetamines, barbiturates, diazepines	Identification in urine	μ Bondapak C18	300x4	MeOH-(aq. 125ml 0.1M KH ₂ PO ₄ +20.3ml 0.1M NaOH, dil. to 750 ml with H ₂ O, pH 6.2 with NaOH or H ₃ PO ₄)(6:4) ²	82
P, H, ethaverine, coc, S, caf, yohimbine	Various basic compounds	Determination P in blood	Micropak CN-10	300x4	n-Hexane-CH ₂ Cl ₂ -ACN-propylamine (75:25:25:0 ² :1) ²	97
C, M, P, norC, N-isopropylC, caf	Par, phen, ibu- profen	Determination C in plasma	μ Bondapak C18	300x3.9	MeOH-H ₂ O-H ₃ PO ₄ (21:79:1.5)	108
C, M, H, EtM, norM, M-3- and M-6-glucuronide		Analysis M in plasma and urine	Ultrasphere ODS 5 μ m	150x4.6	ACN-0.01M NaH ₂ PO ₄ buffer pH 2.1 (26:74) containing 0.001M dodecyl- sulfate	110
M	Monoamine transmitters	Determination in mouse brains	Ultrasphere ODS 5 μ m	250x4.6	0.05M Citrate buffer pH 4.25 containing 1% THF	111
M, nalo		Determination in cerebrospi- nal fluid and plasma	μ Bondapak C18	300x4	ACN-MeOH-aq. 0.07M KH ₂ PO ₄ , 0.5mM EDTA(5:8:87)	112
C, M, H, 60AcM, AcC, pxyC, oxyM, diHCone, diHMone, mep, normep, meth, prop, nalo, nalo, lev, Q, caf, coc	Various drugs	Determination H and metabo- lites in blood	Lichrosorb Si60 5 μ m	300x4	ACN-MeOH-(MeOH-NH ₄ OH(2:1))-(AcOH- MeOH(1:1))(75:25:0.040:0.216)	113
C, M, H, 60AcM, norC, norM, AcC, EtM, diHM, M-3-glucuronide, nalo		Determination M in biologi- cal fluids, using fluores- cence detection	Silica gel 60 5 μ m Partisil 10 ODS or Zorbax ODS 8 μ m	200x4 100x4	MeOH-2M NH ₄ OH-1M NH ₄ NO ₃ (3:2:1) MeOH-0.1M KBr(12.5:87.5) pH 3 with H ₃ PO ₄	114
M, nalo, naltrexone		Determination in rat brains	RSil C18HL 10 μ m	250x4.6	MeOH-H ₂ O(1:3) containing 0.05M tetramethylammonium, pH 6.1 with H ₃ PO ₄	115

TABLE 7.18

HPLC ANALYSIS DRUGS OF ABUSE IN DRUG SEIZURES AND AS PURE COMPOUNDS

ALKALOIDS*	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF
H,60AcM,M	Procaine	Quantitation and identification	Porasil T 37-50 μ m	1000x4.5	CHCl ₃ -MeOH(4:1)	1
C,M,P,T,H,coc,Q, cinchonine,caf		Separation by dynamic coating HPLC	Corasil I and II coated with Poly G-300(2%)	1000x1	Heptane-EtOH(10:1) with different % of saturation with Poly G-300	2,3
C,M,No,P,T,H,crypt, 60AcM,meth,caf	Acsal,phen, p-aminophenol, par	Separation by ion-exchange HPLC	Zipax SCX 37-44 μ m	1000x2.1	0.2M NaOH pH 9.5 with boric acid, 0.2M KNO ₃ , 4% ACN, 1% prOH 0.15M NaOH pH 9.8 with boric acid 0.04M NaOH pH 9.3 with boric acid, 12% ACN, 2% prOH	4
M,H,60AcM,meth		Quantitative analysis and identification	Zipax SAX 37-44 μ m	1000x2	0.15M NaOH pH 8.8 with boric acid	5
C,M,No,P,T,H,crypt, Losine,EtM,oxyM,di- HCone,diHMone,apoM, meth,isometh,mep,Q, Qd,coc	Methapyrilene, anileridine, procaine	Analysis drugs of abuse	Zipax SAX	1000x2.1	0.08M NaOH pH 9.5 with boric acid 0.15M NaOH pH 9.8 with boric acid, 2% ACN 0.04M NaOH pH 9.3 with boric acid, 12% ACN, 2% prOH 0.15M NaOH pH 8.8 with boric acid	6
C,H,meth,prop,coc, LSD,S,Q,mesc	Amphetamines, benzodiazepines, barbiturates, THC,procaine, various others	Identification streetdrugs	Corasil II 37-50 μ m	500x2.3	Cyclohexane-MeOH-cyclohexylamine (983:15:2), (945:45:1)	11
			Al ₂ O ₃ Woelm B18 18-30 μ m	500x2.3	Cyclohexane-cyclohexylamine(988:2)	11
			Cofasil II 37-50 μ m	500x2.3	A. Skelly B-95% EtOH-dioxane- cyclohexylamine(99.1:50:25:13) B. idem (686:100:200:14) linear gradient A to B	18
M,H,0AcM,S,caf,Q,LSD		Analysis street drugs	no details		no details	15
C,M,H,60AcM,diHC, caf,S,Q,eph,coc	Barb,par,lig- nocaine,proca- ine	Analysis illicit H samples (Fig.7.2)	Zipax SCX	1200x2.1	A. 0.2M boric acid pH 9.3 with NaOH B. 0.2M boric acid pH 9.8 with NaOH, 12% ACN, 2% prOH linear gradient 0-100% B (2ml/min, 6 min)	18

*For abbreviations see footnote at the end of this Table

C,M,Na,No,P,T,H,cot, Losine,prot,EtM,mep, oxyC,diHC,diHM,oxyM, diHCone,diHMone,Ac-diHCone,60AcM,nalo, A,coc,caf,nic,Q,Qd, S,Tp,LSD,isoLSD, Lysac,Lysam	Amphetamines, diazepam, local anaesthetics, analgesics, various others	Separation various drugs of abuse (Table 7.8, 7.9 and Fig.7.16)	Partisil 6 μ m	250x4.6	MeOH-2M NH ₄ OH-1M NH ₄ NO ₃ (27:2:1) MeOH-0.2M NH ₄ NO ₃ (3:2)	
M,H,met,h,coc		Screening drugs of abuse	Bondapak C18/Corasil	610x2	ACN-H ₂ O(9:1), (65:35), (1:1) all with 0.1% by weight (NH ₄) ₂ CO ₃	22
M,mep,coc,nic,eph,Q, caf,tubocurarine	Amphetamines, analgesics, sulfas, barbiturates	Evaluation ion-exchange and reversed-phase columns for analysis drugs	Partisil SCX 10 μ m	250x4.6	(NH ₄)H ₂ PO ₄ buffers pH 3, 5 or 7, ionic strength 0.5, 0.1, 0.05 or 0.01M, with 0, 20, 40 or 60% MeOH	24
			μ Bondapak C18	300x4	0.025M NaH ₂ PO ₄ or Na ₂ HPO ₄ buffers pH 3, 5, 7 or 9, with 0, 20, 40, 60 or 80% MeOH	21,30
C,M,No,P,EtM,coc		Identification	Zipax SCX		0.2M NaOH+5% proH, 1% KNO ₃ and 2% ACN (pH 9)	34
C,M,No,P,T,H,OAcM, AcC,Q,S,Tp,caf,coc, ergot alkaloids	Amphetamines, barbiturates, local anaesthetics	Ion-pair HPLC of drugs of abuse (Table 7.3)	μ Bondapak C18	300x4	0.005M Heptanesulfonic acid in MeOH-AcOH-H ₂ O(40:1:59) (pH 3.5)	38,73
M,Na,P,H,AcC,60AcM, caf		Determination in street drugs	μ Bondapak C18	300x4	ACN-H ₂ O-1% aq. (NH ₄) ₂ CO ₃ (140:156:4)	44
M,H,OAcM,AcC,Q	Procaine	Identification street drugs	Partisil 10 ODS	250x4	ACN-H ₂ O(7:3), (6:4) containing 0.1% (NH ₄) ₂ CO ₃	47
C,M,No,P,H,diHC,EtM, diHCone,diHMone,nic, oxyC,oxyM,met,h,caf, Tb,Tp,eph,mesc,coc, rest Q,S,lobeline	totally 101 drugs of forensic interest	Identification by means of dual wavelength detection (Table 2.2, 2.3)	μ Bondapak C18	300x3.9	0.025M NaH ₂ PO ₄ in MeOH-H ₂ O(2:3) pH 7.0	
			μ Porasil	300x3.9	MeOH-2M NH ₄ OH-1M NH ₄ NO ₃ (27:2:1) CH ₂ Cl ₂ -conc. NH ₄ OH(1000:2)	56
M,H,60AcM,30AcM		Separation H from its hydrolysis products	μ Bondapak C18	300x3.9	ACN-aq. 0.015M KH ₂ PO ₄ (3:7) (pH 3.5)	57
C,M,P,H,AcC,OAcM,oxyC, oxyM,diHCone,diHMone, noroxyM,nalo		Separation of illicit samples (Table 7.5)	μ Bondapak C18	300x4	MeOH-aq. 0.01M Bu ₄ N pH 7.5(47:53) MeOH-aq. 0.01M heptanesulfonic acid pH 3 (35:65) changing to(55:45) after 20 ml	64
C,M,H,EtM,oxyC,oxyM, diHC,diHCone,diHMone, lev,dmethphan,pentazocine,levallorphan,phenazocine		Estimation retention indices from Hansch substituent constants	μ Bondapak C18	300x3.9	MeOH-H ₂ O(2:3) containing 1.65 g K ₂ HPO ₄ and 2.1g KH ₂ PO ₄ /l MeOH-H ₂ O(7:3) containing 0.82 g K ₂ HPO ₄ and 1.05g KH ₂ PO ₄ /l	74

C,M,No,P,T,H,AcC OAcM,caf,S		Analysis illicit heroin samples	μBondapak C18	300x4	ACN-0.75% aq. NH ₄ OAc(65:35)	79
C,M,Na,No,P,T,narcoto- line,laudanidine,oxydi- morphine		Separation	Bondapak CX Corasil Bondapak C18 Corasil P-cellulose		ACN-0.1N aq. Ca-phosphate buffer pH 4.8 (3:7) ACN-0.1N aq. Ca-phosphate buffer pH 7.5 (3:7) 0.1N Na-phosphate buffer pH 7.5	87
C,M,No,P,H,60AcM, AcC,mep, meth, caf, coc,Q,Qd,S	Hypnotics, ana- lgesics, local anaesthetics	Analysis heroin seizures (Table 7.11)	μPorasil	300x4	Cyclohexane-(CHCl ₃ -MeOH-NH ₄ OH (800:200:1))(3:1) ³	91
C,M,No,P,T,H,AcC,EtM, 30AcM,60AcM,caf,Q,S		Analysis illicit heroin samples(Fig.7.6)	S5 NH ₂ , aminopropyl	250x4	ACN-0.005M tetrabutyl ammonium phosphate(85:15)	93
C,M,No,P		Statistical optimization method for HPLC system development	μBondapak C18	300x4	0.001M Phosphate buffer and 0.015M camphorsulfonic acid in MeOH-H ₂ O (41:59)(pH 2.0)	94
C,M,No,P,T,norM		Ion-exchange and straight- -phase partition HPLC on silica gel (Fig. 7.17)	Lichrosorb Si60 5 μm	150x4.6	ACN-H ₂ O-AcOH-DEA(10:90:0.5:0.5) THF-MeOH-AcOH-DEA-H ₂ O(80:20:0.5: 0.5:1)	95
M,nalox,lev,ethorphine		Detection by means of fluor- escence	Supelco LC18	150x4.6	ACN-aq. phosphate buffer pH 5.05 (65:35)	96
C,M,No,P,T,H,AcC, AcM,caf,Tp,coc,eph, S,Q,Qd,mesc,LSD, isoLSD	Barbiturates, amphetamines, local anaes- thetics	Ion-pair HPLC of drugs of forensic interest	μBondapak C18, μBondapak Phenyl or μBondapak CN	300x3.9	0.005M Alkylsulfonate (C ₁ ,C ₂ ,C ₃) in MeOH-H ₂ O-AcOH(40:59:1),(30:69:1), (20:79:1)(pH 3.5)	98, 99,100
M,No,P,H,60AcM,AcC	Procaine,Acsal Ac-procaine, ascorbic acid	Detection Ac-procaine in heroin(Fig.7.8)	μBondapak C18	300x4	ACN-H ₂ O(91.5:8.5) containing 0.008% tris(hydroxymethyl)aminomethane	105
M,P,T,H,60AcM,AcC, caf	Procaine	Multichannel detection in analysis heroin samples	APS-hypersil 5 μm	100x5	ACN-0.005M tetrabutylammonium phosphate (85:15)	106
H		Analysis street samples	no details available			107
T,H,AcC,caf		Analysis illicit heroin sam- ples with GCMS and HPLC	C8-silica gel 10 μm	250x4.6	ACN-0.75% NH ₄ OAc(55:45)	120
C,M,No,P,T,H,AcC, AcM,mep, meth, caf, Tp, A,Q,S,eph,coc,tropa- coc	Analgesics,an- tihistaminics, hypnotics,lo- cal anaesthetics, various others	Analysis heroin seizures (Table 7.6)	μBondapak C18 or Partisil 10-ODS-3	300x3.9 250x4.6	ACN-H ₂ O-H ₃ PO ₄ (12:87:1) containing 0.02M methanesulfonic acid pH 2.2	

* Abbreviations used in Tables 7.13-7.18

A	Atropine	Mesc	Mescaline
AcC	Acetylcodeine	Meth	Methadone
AcDiHCone	Acetyldihydrocodeinone(thebacone)	Na	Narceine
Acsal	Acetylsalicylic acid	Nalo	Nalorphine
ApoC	Apocodeine	Nalox	Naloxone
ApoM	Apomorphine	Nic	Nicotine
B	Brucine	No	Noscapine
Barb	Barbital	30AcM	3-O-Acetylmorphine
C	Codeine	60AcM	6-O-Acetylmorphine
Caf	Caffeine	OxyC	Oxycodone
Coc	Cocaine	OxyM	Oxymorphone
Cot	Cotarnine	P	Papaverine
Crypt	Cryptopine	Par	Paracetamol
DiHC	Dihydrocodeine	Phb	Phenobarbital
DiHCone	Dihydrocodeinone	Phen	Phenacetine
DiHM	Dihydromorphine	Prop	Propoxyphene
DiHMone	Dihydromorphinone	Prot	Protopine
Dmethphan	Dextromethorphan	PseudoM	Pseudomorphine
Eph	Ephedrine	Q	Quinine
EtM	Ethylmorphine	Qd	Quinidine
H	Heroin	S	Strychnine
Keto	Ketobemidone	Salam	Salicylamide
Lev	Levorphanol	Scop	Scopolamine
Losine	Laudanosine	T	Thebaine
M	Morphine	Tb	Theobromine
Mep	Meperidine	Tp	Theophylline

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Chapter 8

TERPENOID INDOLE ALKALOIDS AND SIMPLE INDOLE ALKALOIDS

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The indole alkaloids form the greatest group of alkaloids. Several hundreds of alkaloids containing an indole skeleton are known so far. Rich in indole alkaloids are the plant families Apocynaceae, Loganiaceae and Rubiaceae. Many of the indole alkaloids have characteristic pharmacological activities and are, therefore, used in therapy.

HPLC has been extensively applied in the analysis of indole alkaloids - for quite different purposes. A series of papers on the analysis of drugs of abuse includes the analysis of one or more indole alkaloids, particularly strychnine, a common adulterant of heroin (Table 8.16). Moreover strychnine and brucine have often been used as test compounds for a number of separation systems^{1,2,9,16,21,32,40,43} as well as internal standards^{10,13}.

Comparison of HPLC and TLC in the analysis of indole alkaloids has been performed^{4,27,31,34,41,44}, as well as that for HPLC and GLC⁴. Semipreparative aspects of HPLC in the isolation of indole alkaloids have been investigated^{17,44}. Because of the poor volatility and instability of many high molecular indole alkaloids, such as reserpine and psilocybin, HPLC lends itself to the analysis of such alkaloids, as compared with GLC^{6,11,20,23,27,30,37,46}.

A review on the identification of drugs of abuse, including *Psilocybe* alkaloids, has been given by Gough and Baker⁶⁸.

8.1. ION-EXCHANGE HPLC

Rodgers⁶ used a pellicular strong cation-exchange packing for the separation of some *Rauwolfia* alkaloids. An ammonium phosphate buffer (pH 7.0) containing methanol was used as eluent. An improved separation was obtained with gradient elution.

Walton and Murgia^{9,16,21} described ligand-exchange chromatography of alkaloids on ion-exchange materials loaded with metal ions (see Chapter 7). Perkal et al.⁴⁶ separated psilocybin and psilocin on a cation-exchanger - Partisil SCX - using methanol - water (1:4) containing 0.2% ammonium phosphate and 0.1% potassium chloride (pH 4.5) as mobile phase (Fig.8.1).

8.2. REVERSED-PHASE HPLC

Wu and Siggia^{1,2} described a dynamic coating technique for the application of a liquid stationary phase - Poly G-300 - on a silica gel support. With heptane - ethanol (10:1) saturated with the stationary phase, strychnine and brucine could be separated. Polyethylene glycol coated silica gel as support has, however, been superseded by chemically bonded stationary phases.

Chemically bonded octadecyl groups on a pellicular support was used as stationary phase for the separation of a series of *Mitragyna* oxindole alkaloids⁴ with methanol - water (4:1) as mobile phase.

Phillipson et al.⁷⁷ compared the retention behaviour of nine heteroyohimbine and eight oxindole alkaloids on an octadecyl bonded phase with previously obtained results on straight-phase TLC (Table 8.1). If the retention on silica gel could be explained in terms of the availability of the N-4 lone-pair electrons, such a correlation could not be concluded for the retention in the reversed-phase system.

Reserpine has been analyzed in pharmaceutical preparations with diuretic and antihypertensive drugs on pellicular octadecyl or phenyl packings^{11,20}. A series of mobile phases was used that could meet the different analytical demands. In solvent systems with a pH of over 8, several peaks were observed in addition to reserpine. They were assumed to be due to the decomposition of reserpine during the analysis.

For the rapid qualitative and quantitative analysis of therapeutically used *Catharanthus* alkaloids, Görög et al.²⁶ applied reversed-phase HPLC on an octyl type column. With acetonitrile - 0.01 M ammonium carbonate (47:53) a fairly good separation was achieved, both in terms of analysis time and resolution (Table 8.2, Fig. 8.2). *Catharanthus* alkaloids isolated from tissue cultures have been separated on an octadecyl column with methanol - water - triethylamine mixtures⁵⁰.

Verzele et al.⁶⁰ preferred a gradient elution (from 50 to 85% methanol in water containing 0.1% ethanolamine) to separate the wide range of different alkaloids present in *Vinca rosea*. It was found that column efficiency was improved by increasing the temperature to 50°C (doubled plate number if compared with 5°C).

Szepesi and Gazdag⁵⁴ studied the separation of 22 eburnane alkaloids on an octyl and octadecyl type of column. Optimal results were obtained on both columns with the mobile phase acetonitrile - 0.01 M aqueous ammonium carbonate (3:2). The octadecyl stationary phase gave better resolution than the octyl packing (Table 8.3, Fig. 8.3). The reversed-phase system was capable of separating the three different groups of eburnane alkaloids as well as separating the ester homologues and some stereoisomers. Structural and stereoisomers were, however, separated better with straight-phase HPLC systems (see below)⁵⁵. The applications of the developed methods to various separation problems involving eburnane alkaloids have been discussed⁷⁵.

Dubruc et al.⁵³ reported the analysis of vincamine in plasma. Analysis was performed on an octadecyl bonded stationary phase. Large volumes (0.5 ml) of the sample in a non-eluting solvent (0.02 M aqueous potassium phosphate) were used for automatic operation of the analysis. As mobile phase: acetonitrile - acidic phosphate buffer was used. Dilute samples of urine and plasma have been injected directly on an octadecyl column for the determination of the vincamine level⁵⁸. For mobile phase methanol - aqueous ammonium carbonate was employed.

Hussey and Newton³¹ analyzed vindesine by means of a microparticulate octadecyl column and a mobile phase of methanol - water - diethylamine (1000:600:3). For the analysis of vindesine in the presence of high levels of 6,7-dihydrovindesine, the ratio (835:600:3) was preferred. For pharmaceutical preparations containing vindesine, a 0.5 M potassium phosphate buffer in combination with a higher amount of methanol could be used instead of diethylamine to give prolonged column life.

To avoid column degradation due to high pH, Crouch and Short²⁹ analyzed strychnine on an octadecyl column with an acidic mobile phase: methanol - 0.005 M potassium dihydrogen phos-

TABLE 8.1

RETENTION TIMES OF HETEROYOHIMBINE- AND OXINDOLE-TYPE ALKALOIDS⁷⁷

Column, Spherisorb ODS 5 μ m (250x4 mm ID), mobile phase, S1 methanol - water (80:20), S2 methanol - water - conc. ammonia (80:20:1), S3 acetonitrile - 1% ammonium carbonate (60:40), S4 methanol - 1% ammonium carbonate (80:20), flow rate 2 ml/min, detection UV 254 nm.

Alkaloids	t_r (min) in solvent system			
	S1	S2	S3	S4
<i>Pentacyclic heteroyohimbines</i>				
Tetrahydroalstonine	5.1	4.8	6.2	3.6
Rauneticine	10.9	8.6	10.0	10.0
Akuammigine	5.8	4.6	6.5	4.2
3-Iso-rauneticine	1.0	1.0	-	-
Ajmalicine	4.3	3.4	5.2	2.9
3-Iso-ajmalicine	2.0	2.0	-	-
19-Epi-3-isoajmalicine	1.6	1.6	-	-
<i>Tetracyclic heteroyohimbines</i>				
Dihydrocorynantheine	7.7	3.9	-	3.7
Hirsutine	12.7	4.9	-	-
<i>Pentacyclic oxindoles</i>				
Isopteropodine	3.8	3.8	3.7	2.9
Pteropodine	3.8	3.2	2.8	2.3
Spectophylline	3.7	3.0	2.8	2.2
Uncarine F	3.7	3.0	2.7	2.2
Mitraphylline	3.4	3.2	3.0	2.4
Isomitraphylline	3.7	3.0	2.7	2.0
<i>Tetracyclic oxindoles</i>				
Isorhynchophylline	3.0	3.0	3.3	3.3
Rhynchophylline	6.2	4.0	5.1	4.2

TABLE 8.2

RETENTION DATA FOR SOME CATHARANTHUS ALKALOIDS AND SEMISYNTHETIC DERIVATIVES (Fig.8.2)²⁶

Column, Lichrosorb RP8 (250x4 mm ID), mobile phase, acetonitrile - 0.01 M ammonium carbonate (47:53), flow rate 1.5 ml/min, detection UV 298 nm.

Alkaloids	Substituted at			Retention time (min)
<i>Monomeric alkaloids</i>				
Lochnerine				3.29
Ajmalicine				7.90
Tetrahydroalstonine				14.65
Catharanthine				9.75
<i>Vindoline derivatives</i>	16 β	16 α	17	
Vindoline	OH	COOCH ₃	OCOCH ₃	6.16
	OH	COOCH ₃	OH	4.71
	OH	COOCH ₃	OCOCH ₂ Cl	9.39
	OH	COOCH ₃	OCOCH ₂ N(CH ₃) ₂	5.84
	OH	COOCH ₃	OCOCH ₂ NH(CH ₃)	4.30
	OCOCH ₃	COOCH ₃	OCOCH ₃	23.22
Vindolinol	OH	CH ₂ OH	OH	4.44
Vindorosine				7.90
<i>Dimeric alkaloids</i>				
<i>Vinblastine and derivatives</i>	N	16 β	17	
Vinblastine	CH ₃	OH	OCOCH ₃	12.37
	CH ₃	OH	OH	7.04
	CH ₃	OH	OCOCH ₂ Cl	12.52
	CH ₃	OH	OCOCH ₂ N(CH ₃) ₂	10.25
	CH ₃	OH	OCOCH ₂ NH(CH ₃)	10.09
	CH ₃	OCOCH ₃	OCOCH ₃	48.10
	H	OH	OH	6.18
	H	OH	OCOCH ₃	10.04
Desacetoxyvinblastine	CH ₃	OH	H	17.15
Vincristine	CHO	OH	OCOCH ₃	7.22
	CHO	OH	OH	4.87
Leorosine	CH ₃			15.65
	H			11.89
Formylleurosine	CHO			9.75

TABLE 8.3

CAPACITY RATIOS, k' , FOR EBURNANE ALKALOIDS ON OCTADECYL AND OCTYL SILICA PACKINGS WITH DIFFERENT ELUENTS⁵⁴Column μ Bondapak C18 (300x3.9 mm ID) or 10 μ m Lichrosorb RP8 (250x4.6 mm ID), flow rate 1 ml/min, detection UV 280 nm.

Alkaloid	Ratio of acetonitrile and 0.01 M (NH ₄) ₂ CO ₃									
	4:6	5:5	6:4	7:3	8:2	4:6	5:5	6:4	7:3	
	μBondapak C18					10 μm LiChrosorb RP-8				
(+)-cis-Apovincaminic acid	0.05	0.05	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
(+)-cis-Vincaminic acid	0.10	0.10	0.07	0.05	0.0	0.0	0.0	0.0	0.0	
(+)-cis-Dehydroepivincamine	4.65	2.57	1.57	1.04	0.57	4.61	2.14	1.30	0.66	
(+)-cis-Dehydrovincamine	6.03	3.21	1.86	1.31	0.57	5.65	2.60	1.55	0.98	
(+)-trans-Vincaminic acid ethyl ester	8.93	4.29	2.29	1.32	0.57	10.81	4.43	2.19	1.30	
(+)-cis-Epivincamine	9.23	4.71	2.71	1.79	0.75	6.02	2.60	1.55	1.04	
(-)-cis-Epivincamine	9.23	4.71	2.71	1.79	0.75	6.02	2.60	1.55	1.04	
(+)-cis-Epivincaminic acid ethyl ester	12.4	6.36	3.43	1.97	0.95	8.45	3.60	1.92	1.30	
(+)-trans-Epivincaminic acid ethyl ester	14.4	6.36	3.43	1.79	0.75	19.8	7.04	3.26	1.83	
(+)-cis-Vincamine	12.4	6.36	3.43	2.14	0.90	10.9	3.53	1.87	1.30	
(-)-cis-Vincamine	12.4	6.36	3.43	2.14	0.90	10.9	3.53	1.87	1.30	
(+)-cis-Vincamone	13.7	7.21	4.04	2.57	1.43	-	5.51	3.19	1.87	
(+)-cis-Vincanole	15.6	8.07	4.29	3.14	1.71	-	3.60	2.26	1.55	
(+)-cis-Isovincanole	18.8	9.43	5.43	3.71	1.71	-	4.30	2.83	1.87	
(+)-cis-Vincaminic acid ethyl ester	16.9	8.47	4.38	2.57	1.43	12.4	5.03	2.57	1.55	
(+)-cis-Apovincamine	23.9	14.1	6.78	4.11	2.64	-	9.42	4.79	2.66	
(+)-trans-Apovincaminic acid ethyl ester	30.3	18.5	7.76	3.97	2.03	-	21.8	8.64	4.23	
(+)-cis-Apovincaminic acid ethyl ester	32.5	20.2	9.00	6.20	3.27	-	13.6	6.36	3.47	
(+)-cis-Vincamenine	46.5	31.2	14.0	8.71	5.57	-	19.2	10.4	5.26	
(+)-cis-Apovincaminic acid phenyl ester	56.1	36.8	14.0	7.07	4.00	-	26.4	9.80	4.81	
(+)-cis-10-Bromovincamine	13.4	9.11	4.86	3.54	1.70	-	7.32	3.57	2.11	
(+)-cis-11-Bromovincamine	13.4	9.11	4.86	3.54	1.70	-	7.32	3.57	2.11	

TABLE 8.4

SEPARATION OF SOME ALKALOIDS ON A POROUS POLYMER⁴³

Column, Hitachi Gel 3010 (macroporous styrene-divinylbenzene copolymer), 10 μ m (22x4.6 mm ID), mobile phase, S1 acetonitrile - water (3:7) containing 0.02 M tetrabutylammonium hydroxide, S2 acetonitrile - water (6:4) containing 0.02 M tetrabutylammonium hydroxide, S3 acetonitrile - water (6:4) containing 0.02 M ammonia, flow rate 1 ml/min, detection UV 254 nm.

Compound	k' in S1 (55° C)	Compound	k' in S2 (25° C)
Tyramine	0.4	Yohimbine	2.6
Norephedrine	0.8	Reserpine	11.7
Ephedrine	1.8		
Atropine	3.9		k' in S3 (65° C)
Amphetamine	4.0	2,4,6-Trimethylpyridine	3.2
Methamphetamine	7.3	2-Methylquinoline	4.5
Strychnine	8.4	5,6-Dibenzoquinoline	12.5
Cinchonine	10.4	Acridine	13.0
Quinine	12.6		

phate buffer (pH 3.0)(2:3). Strychnine and brucine were not separated in this system.

Sasse et al.⁴⁵ reported the analysis of harmaline alkaloids in cell suspension cultures by means of HPLC. An octyl column and a mobile phase of methanol - water - formic acid (166:34:1) buffered at pH 8.5 with triethylamine was used for the separation of the alkaloids (Fig.8.4).

For the analysis of ellipticine in biological samples an octadecyl stationary phase in combination with acetonitrile - 0.01 M sodium dihydrogenphosphate buffer has been used⁷⁰. To ensure resolution between ellipticine and 9-hydroxyellipticine a ratio of 1:3 had to be used (Fig.8.5). In the case where only ellipticine is present, a ratio of 36:64 was used.

Tymes⁶⁷ reported a collaborative study of the analysis of physostigmine in pharmaceutical preparations using octadecyl stationary phases with the mobile phase: acetonitrile - 0.05 M ammonium acetate (1:1).

Aramaki et al.⁴³ made a preliminary study of the separation of some alkaloids on a macroporous styrene-divinylbenzene polymer (Table 8.4, Fig.8.6). An advantage of these columns is their stability, also under the strong basic conditions applied for the analysis of alkaloids. It was assumed that alkaloids would be best retained under basic conditions, the alkaloids being in the uncharged, non-protonated form.

8.3. ION-PAIR HPLC

Reversed-phase ion-pair chromatography has been applied for the analysis of drugs of abuse - including strychnine^{24,63,64,65,82}. For a more detailed discussion see Chapter 7 (Table 7.6).

Sams²⁷ reported the determination of reserpine in plasma by ion-pair chromatography. To obtain high specificity and to increase the sensitivity reserpine - after extraction from plasma with benzene - was oxidized to 3-dehydroreserpine with vanadium pentoxide in concentrated phosphoric acid, to which 9 ml methanol were added. The fluorescent compound was subsequently analyzed on an octadecyl column with methanol - 0.001 M heptanesulfonate in water (65:35) as mobile phase. Reserpine and rescinnamine could not be distinguished by this method.

Ellipticine and its derivatives as well as related quaternary ammonium compounds have been separated on an octadecyl column with methanol - water mixtures, to which 1-heptanesulfonate

TABLE 8.5.

Structures and retention times of six ellipticines³⁶Column μ Bondapak C18 (300x4 mm ID), mobile phases:

S1 methanol - water (7:3)

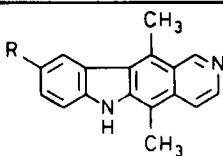
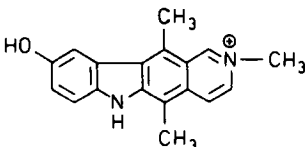
S2 methanol - water (7:3) containing 0.005 M 1-heptanesulfonate - 0.032 M acetic acid

S3 methanol - water (7:3) containing 0.005 M 1-pentanesulfonate - 0.032 M acetic acid

S4 methanol - water (3:1) containing 0.005 M 1-heptanesulfonate - 0.032 M acetic acid

S5 methanol - water (3:1) containing 0.02 M ammonium acetate

Flow rate 1.2 ml/min, detection UV 254 nm and 313 nm, fluorescence (excitation 305 nm, emission 470 nm).

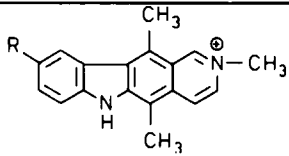
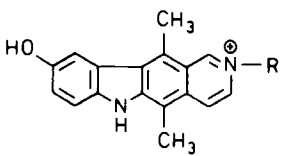
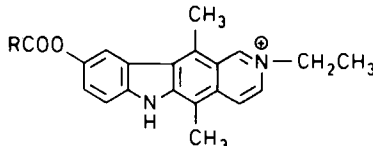
Compound	R	t_r (min)				
		S1	S2	S3	S4	S5
	NH ₂	>30	3.8	3.5	3.5	4.9
	OH	>30	4.5	3.8	4.0	5.5
	H	>30	7.1	5.2	5.4	9.3
	OCH ₃	>30	7.4	5.7	5.7	9.5
	Br	>30	13.5	8.2	8.2	-
	- **	>30	4.6	3.9	4.1	4.2

* Ellipticine

** 9-Hydroxy-2-methylellipticinium

TABLE 8.6.

Structures and retention times of some ellipticine quaternary ammonium derivatives³⁶Column μ Bondapak C18 (300x4 mm ID), mobile phase methanol - water (75:25) containing 0.005 M sodium 1-heptanesulfonate and 0.032 M acetic acid, flow rate 1.2 ml/min, detection UV 254 nm and 313 nm, fluorescence (excitation 305 nm, emission 470 nm).

Compound	R	t_r (min)
	H	5.7
	NH ₂	3.7
	OCH ₃	6.0
	OCOCH ₃	5.1
	NO ₂	5.6
	Br ²	8.4
	CH ₃	4.1
	C ₂ H ₅	4.5
	n-C ₃ H ₇	5.1
	n-C ₄ H ₉	5.9
	n-C ₅ H ₁₁	7.6
	CH ₂ =CHCH ₂	4.7
	CH ₂ CH ₂ OH	3.4
	R ₁	4.3
	R ₂	4.6
	R ₃	4.8
	CH ₃	5.4
	n-C ₃ H ₇	8.7
	n-C ₅ H ₁₁	16.0
	C ₆ H ₅	11.8
	C ₆ H ₅	11.8

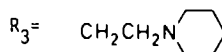
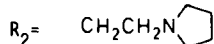
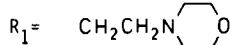


TABLE 8.7

HPLC ANALYSIS PILOCARPINE, PHYSOSTIGMINE, ITS DEGRADATION PRODUCTS AND PRESERVATIVES IN PHARMACEUTICAL PREPARATIONS⁴⁷

Column, μ Bondapak C18 (300x3.9 mm ID), mobile phase, methanol - water (2:3) containing 0.005 M heptanesulfonic acid (pH 3.6), flow rate 1 ml/min, detection UV 235 nm and 292 nm (for rubreserine).

Compound	k'	Sample conc. (μ g)	Detection limit (μ g)
Rubreserine	1.7	0.02	0.001
Pilocarpine	2.3	80	0.02
Salicylate	2.9	8	-
Phenethyl alcohol	3.5	10	-
Methyl p-hydroxybenzoate	4.2	0.8	-
Physostigmine	5.1	8	0.003

was added as counter ion (Table 8.5, 8.6)³⁶. Ammonium acetate was used as ionic component in the mobile phase.

Kneczek⁴⁷ analyzed pharmaceutical preparations containing pilocarpine and physostigmine as well as their degradation products and preservatives on an octadecyl column using ion-pair chromatography (Table 8.7, Fig.8.7).

Thomson⁴⁹ reported the reversed-phase ion-pair separation of psilocybin and psilocin. Because both alkaloids exist as zwitter-ions, cationic and anionic pairing ions can be used. Alkylsulfonates (C_5 - C_8) and tetraalkyl ammonium (C_3 - C_6) ions were found unsatisfactory for psilocybin. Good results were obtained with a long chain quaternary ammonium ion, cetrimonium. Optimal conditions for quantitative analysis on an octadecyl stationary phase were 0.15% pairing ion in methanol - 0.4% aqueous phosphate buffer (pH 7.2). Some other quaternary indole alkaloids have also been separated by means of ion-pair HPLC. Parkin⁶¹ analyzed the bisquaternary alkaloid alcurnonium in biological fluids. After an ion-pair extraction, the alkaloid was analyzed on an octadecyl column with the mobile phase: methanol - water (4:1) containing 0.25% acetic acid and 0.005 M dodecylsulfate.

N-propylajmaline has been analyzed by means of straight-phase ion-pair HPLC. A silica gel column loaded with 0.2 M perchloric acid and 0.8 M sodium perchlorate in combination with the mobile phase: n-butanol - 1,2-dichloroethane - hexane (15:40:45) was suitable for separating the two C-21 epimers, as extracted from plasma⁸³.

Szepesi et al.⁷³ reported an ion-pair separation of eburnane alkaloids on a chemically bonded cyanopropyl stationary phase. As counter-ion, di-(2-ethylhexyl)phosphoric acid or (+)-10-camphorsulfonic acid were used in a mobile phase consisting of hexane - chloroform - acetonitrile mixtures (Table 8.8, 8.9). Because of the poor solubility of the latter pairing ion, diethylamine (Table 8.9) was added to the mobile phase. Addition of diethylamine considerably reduced the k' of the alkaloids, due to suppression of the ionization of the alkaloids. However, due to the strong acidic character of the pairing ion, ion-pairs were still formed under these conditions. The camphorsulfonic acid containing mobile phases were found to be very useful for the separation of optical isomers (Table 8.10, 8.11, Fig.8.8)⁷⁶. It was also found that the selectivity of the system could be altered by choosing different medium-polarity solvents (moderator solvents) as dioxane, chloroform or tetrahydrofuran. The polar component of the solvent system affected peak shape. Based on these observations, a method was developed to analyze the optical purity of vincamine and vinpocetine. For the ana-

TABLE 8.8

DEPENDENCE OF THE CAPACITY RATIOS (k') MEASURED FOR EBURNANE ALKALOIDS ON THE DI-(2-ETHYL-HEXYL)PHOSPHORIC ACID (DHP) CONCENTRATION³

Column, μ Bondapak CN (300x3.9 mm ID), eluent flow rate 1 ml/min, detection UV 280 nm.

Compound	Eluent composition(%)								
	Hexane	65	65	65	65	65	65	60	70
	Chloroform	20	20	20	20	20	20	23	17
	Acetonitrile	15	15	15	15	15	15	17	13
	DHP (mol/l)	-	0.0005	0.001	0.005	0.01	0.025	0.005	0.005
Hydrocortisone	1.89	1.89	1.97	1.92	1.93	1.94	1.48	3.00	
Prednisolone	2.52	2.50	2.52	2.53	2.55	2.59	1.89	3.90	
(+)-cis-Epivincamine	2.93	2.36	4.07	2.36	2.31	3.44	1.96	3.00	
(+)-cis-Vincamine	1.93	1.75	5.34	6.57	7.46	9.04	5.78	10.1	
(+)-cis-Apovincaminic acid ethyl ester	0.45	0.43	2.24	3.36	4.25	4.78	2.78	4.26	
(+)-cis-Vincamenine	0.93	0.86	3.00	3.64	3.92	4.78	3.30	4.56	
(+)-cis-Apovincamine	0.55	0.54	2.72	4.14	4.42	5.30	3.56	5.44	
(+)-cis-Vincamome	0.28	0.32	1.62	3.50	4.12	4.63	3.19	4.70	
(+)-cis-Vincanol	3.34	2.75	5.83	4.21	3.58	6.33	3.67	5.07	
(-)-cis-Vincanol	3.34	2.75	5.83	4.21	3.58	6.33	3.67	5.07	
(+)-cis-Vincaminic acid ethyl ester	1.52	1.54	4.14	6.50	5.43	6.93	4.78	7.19	
(+)-cis-Isovincanol	5.72	4.18	8.69	10.0	9.08	10.4	7.52	12.7	

lysis of vincaminic acid in pharmaceutical preparations, acetonitrile - aqueous 0.001 M ammonium carbonate (7:3) has been used, containing 0.001 M trioctylmethylammonium as pairing ion⁷⁵.

8.4. STRAIGHT-PHASE HPLC

For strong basic alkaloids such as strychnine, brucine, serpentine and alstonine, the addition of a base to the mobile phase reduces tailing due to chemisorption on the silica gel^{7,41,44}. A mobile phase consisting of diethyl ether - methanol - diethylamine proved to be useful in the separation of *Strychnos* alkaloids on a silica gel column^{7,18} (Table 8.12, Fig.8.9). A similar system was applied by Gimet and Filloux³⁸ for the analysis of various alkaloids in pharmaceutical preparations (Fig.7.14). It was found that an increased saturation of the diethyl ether with water led to reduced retention times; a similar effect was found for the amount of diethylamine added to the solvent. For the separation of *strychnos* alkaloids, good results were also obtained with the mobile phase: ethyl acetate - methanol - ammonia in combination with silica gel columns^{33,41,44}.

Kingston and Li¹⁷ reported for *Tabernaemontana* alkaloids that silica gel in combination with ammonia containing solvent systems or aluminium oxide in combination with neutral solvents could be used for preparative HPLC. The HPLC system described by Bushway et al.¹⁴ - silica gel column and chloroform - methanol - (9:1) as mobile phase - had only limited applicability for the analysis of strychnine, since it did not separate strychnine from brucine.

Ajmalicine (raubasine) analyses have been performed on silica gel with a neutral solvent system - *n*-hexane - diisopropyl ether - methanol (90:10:3)^{28,34}. The HPLC method was found to be more convenient than a TLC densitometric and a UV spectrophotometric method.

TABLE 8.9

DEPENDENCE OF THE CAPACITY RATIOS (k') MEASURED FOR Eburnane Alkaloids ON THE (+)-CAMPHORSULFONIC ACID (CSA) CONCENTRATION⁷³

Conditions as in Table 8.8.

Compound	Eluent mixtures (hexane-isopropanol, 8:2)																
	DEA (M)	0.001	0.001	0.001	0.001	0.001	0.001	-	0.00025	0.0005	0.001	0.002	0.0021	0.00215	0.0022	0.003	0.004
	CSA (M)	-	0.0005	0.001	0.0015	0.003	0.005	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Hydrocortisone		1.97	1.95	1.95	2.05	2.01	2.00										
Prednisolone		2.29	2.18	2.19	2.23	2.26	2.30	2.20	2.27	2.13	2.23	2.16	2.23	2.11	2.20	2.23	2.23
(+)-cis-Epivincamine		0.82	1.21	6.08	7.32	6.75	6.23	7.10	6.60	6.33	6.03	6.00	5.81	5.97	2.26	0.94	0.87
(-)-cis-Epivincamine		0.82	1.21	6.75	8.14	7.64	7.00	7.87	7.30	7.10	6.70	6.67	6.48	6.68	2.26	0.94	0.87
(+)-cis-Vincamine		0.65	1.00	8.23	11.1	10.1	8.97	10.9	10.0	9.50	8.93	8.73	8.48	8.77	1.65	0.74	0.65
(-)-cis-Vincamine		0.65	1.00	8.94	11.5	10.5	9.40	11.4	10.4	9.93	9.33	9.10	8.87	9.10	1.65	0.74	0.65
(+)-cis-Apovincaminic acid ethyl ester		0.41	0.59	6.50	7.46	6.89	6.20	7.20	6.53	6.80	6.23	6.00	5.65	5.45	0.55	0.42	0.42
(-)-cis-Apovincaminic acid ethyl ester		0.41	0.59	6.50	7.46	6.89	6.20	7.20	6.53	6.80	6.23	6.00	5.65	5.45	0.55	0.42	0.42
(+)-trans-Apovincaminic acid ethyl ester				13.2	17.0	16.5	14.5										
(-)-trans-Apovincaminic acid ethyl ester				13.6	17.6	17.2	15.1										
(+)-cis-Vincamenine		0.15	0.31	5.52	6.57	5.71	5.20										
(+)-cis-Apovincamine		0.41	0.66	7.80	8.93	7.75	7.10										
(+)-cis-Vincamone		0.32	0.52	5.71	7.14	6.71	5.97										
(-)-cis-Vincamone		0.32	0.52	5.71	7.14	6.71	5.97										
(+)-cis-Vincanol		0.53	0.62	5.28	7.25	6.75	6.00										
(-)-cis-Vincanol		0.53	0.62	5.71	7.50	7.04	6.17										
(+)-cis-Vincaminic acid ethyl ester		0.47	0.72	8.35	8.71	8.50	7.30										
(-)-cis-Vincaminic acid ethyl ester		0.47	0.72	8.72	9.21	9.04	7.70										

TABLE 8.10

SEPARATION OPTICAL ISOMERIC EBURNANE ALKALOIDS; DEPENDENCE OF CAPACITY FACTORS (k') AND SEPARATION FACTORS (r_{ji}) ON THE CONCENTRATION OF CHLOROFORM AND ALCOHOLS IN THE ELUENT⁷⁶

Column, Nucleosil 10 CN (250x4.6 mm I.D.); flow-rate: 1 ml/min; detection at 280 nm. A 1 l volume of eluent contains 2×10^{-3} mole of (+)-10-camphorsulfonic acid and 10^{-3} mole of DEA.

Compound	Eluent mixture											
	Hexane-chloroform-methanol						Hexane-chloroform-ethanol					
	80:18:2		70:27:3		60:36:4		80:18:2		70:27:3		60:36:4	
	k'	r_{ji}	k'	r_{ji}	k'	r_{ji}	k'	r_{ji}	k'	r_{ji}	k'	r_{ji}
(+)-cis-Vincaminic acid ethyl ester	5.24		1.55		0.65		6.55		1.90		0.75	
(-)-cis-Vincaminic acid ethyl ester	5.50	1.05	1.65	1.06	0.65	1.00	7.20	1.10	2.10	1.11	0.83	1.10
(+)-cis-Epivincamine	5.50	1.05	1.70	1.05	0.72	1.04	7.16	1.10	2.10	1.10	0.90	1.11
(-)-cis-Epivincamine	5.80		1.79		0.75		7.88		2.30		1.00	
(+)-cis-Vincamine	7.29	1.07	2.10	1.05	0.82	1.05	8.90	1.08	2.60	1.08	1.04	1.09
(-)-cis-Vincamine	7.81		2.25		0.86		9.62		2.80		1.14	
(+)-trans-Epivincamine	17.9	1.03	5.15	1.03	1.64	1.00	>20	1.06	8.00	1.06	2.57	1.04
(-)-trans-Epivincamine	18.4		5.30		1.64		>20		8.45		2.67	
(+)-trans-Vincamine	>20		5.30		1.82		>20		10.3		3.38	
(-)-trans-Vincamine	>20		5.50		1.82		>20		10.8		3.57	
(+)-cis-Vincamone	6.50	1.00	1.90	1.00	0.45	1.00	6.14	1.00	2.40	1.00	1.00	1.00
(-)-cis-Vincamone	6.50		1.90		0.45		6.14		2.40		1.00	
(+)-trans-Vincamone	15.5	1.06	3.95	1.04	1.36	1.00	17.9	1.07	7.00	1.07	2.81	1.07
(-)-trans-Vincamone	16.4		4.10		1.36		19.2		7.50		3.00	
(+)-cis-Apovincaminic acid ethyl ester	3.86	1.00	1.20	1.00	0.45	1.00	5.38	1.04	1.60	1.00	0.55	1.00
(-)-cis-Apovincaminic acid ethyl ester	3.86		1.20		0.45		5.57		1.60		0.55	
(+)-trans-Apovincaminic acid ethyl ester	11.0	1.03	2.60	1.00	0.91	1.00	18.7	1.04	4.85	1.03	1.95	1.00
(-)-trans-Apovincaminic acid ethyl ester	11.4		2.60		0.91		19.5		5.00		1.95	
Hydrocortisone	10.3		3.40		1.36		12.5		3.70		1.76	
Prednisolone	14.2		4.60		2.09		17.2		5.10		2.38	

TABLE 8.11

OPTIMAL SEPARATION SYSTEMS FOR OPTICAL ISOMERIC EBURNANE ALKALOIDS⁷⁶

Column, I Nucleosil CN 5 μ m (150x4.6 mm ID), II Nucleosil CN 10 μ m (250x4.6 mm ID), mobile phase, A hexane - dioxane - n-butanol (70:25:5), B hexane - chloroform - ethanol (70:27:3), C hexane - dioxane - ethoxyethanol (57.5:37.5:5), all three solvent systems contain 0.002 M (+)-10-camphorsulfonic acid and 0.001 M diethylamine, flow rate 1.5 ml/min, detection UV 280 nm.

Compound	HPLC system	k'(+)	k'(-)	r _{ji}	H(mm)	R _s	Asf*
(+)-cis-Epivincamine	II - A	7.95	8.70	1.07	0.060	1.35	0.98
	I - B	1.78	1.95	1.10	0.023	1.26	1.15
(+)-cis-Vincamine	II - A	8.90	10.05	1.13	0.064	1.60	1.27
	I - B	2.21	2.37	1.07	0.026	1.16	1.14
(+)-trans-Epivincamine	I - B	7.04	7.42	1.05	0.041	1.14	1.25
(+)-trans-Vincamine	I - B	9.04	9.51	1.05	0.054	1.20	2.08
(+)-cis-Apovincaminic acid ethyl ester	II - A	7.78	8.83	1.14	0.061	1.77	0.92
(+)-trans-Apovincaminic acid ethyl ester	I - B	4.83	4.97	1.03	0.097	1.22	1.44
(+)-cis-Vincamone	II - C	2.83	4.10	1.45	0.084	3.11	1.20
(+)-trans-Vincamone	II - C	10.2	10.8	1.06	0.112	1.15	2.26

* Asf = back part of the peak / front part of the peak

TABLE 8.12

SEPARATION OF SOME *STRYCHNOS* ALKALOIDS¹⁸

Column, Merckosorb Si60 5 μ m (300x2 mm ID), mobile phase S1 diethyl ether - diethyl amine (99:1), flow rate 2.00 ml/min, S2 diethylether - methanol (1:1), flow rate 1.15 ml/min, detection UV 254 nm.

Alkaloid	Retention time (min)		Alkaloid	Retention time (min)	
	S1	S2		S1	S2
Icajine	4.2	2.6	Spermostrychnine	9.8	
Vomicine	4.6	1.6	β -Colubrine	10.3	10.2
Pseudostrychnine	6.8		Diaboline	16.0	10.9
Strychnine	7.2	12.4	Brucine	18.4	17.6
4-Hydroxystrychnine	7.6		Serpentine	>20	
α -Colubrine	8.8	14.3	Alstonine	>20	

TABLE 8.13

RETENTION DATA OF RESERPINE, HYDROCHLOROTHIAZIDE, AND RELATED COMPOUNDS³⁰

Column, Lichrosorb Si60 5 μ m (250x2.1 mm ID), mobile phase n-hexane - isopropanol - chloroform - diethylamine (77:18:5:0.01), flow rate 1.5 ml/min, detection UV 254 nm.

Compound	Retention time (sec)	k'	Compound	Retention time (sec)	k'
Reserpine	68	1.0	3,4,5-Trimethoxycinnamic acid	-	-
3-Isoreserpine	50	0.5	1-Amino-3-chloro-4,6-benzene-		
3,4-Dehydroreserpine	-	-	disulfonamide	210	5.2
3,4,5,6-tetrahydroreserpine(lumi-)	-	-	Hydrochlorothiazide	320	8.5
Deserpidine	61	0.8	Chlorothiazide	200	4.9
Rescinnamine	82	1.4	Methychlothiazide	114	2.4
3,4,5-Trimethoxybenzoic acid	-	-	Bendroflumethiazide	59	0.7
			Polythiazide	108	2.2

* Retained peak

TABLE 8.14

CAPACITY RATIOS, k' , FOR EBURNANE ALKALOIDS USING FOUR-COMPONENT ELUENT MIXTURES⁵⁵

Column Lichrosorb Si60, 5 μ m (250x4.6 mm ID), flow rate 1 ml/min, detection UV 280 nm. Mobile phases hexane - chloroform - acetonitrile - methanol in the ratios 1, (55:20:25:3); 2, (55:25:20:3); 3, (55:22.5:22.5:3); 4, (60:20:20:3); 5, (60:24:15:3); 6, (60:22.5:17.5:3); 7, (65:17.5:17.5:3); 8, (65:20:15:3); 9, (65:15:20:3); 10, (55:25:20:1); 11, (55:25:20:5).

Alkaloid	Eluent										
	1	2	3	4	5	6	7	8	9	10	11
(+)-trans-Apovincaminic acid ethyl ester	0.32	0.18	0.22	0.20	0.15	0.20	0.25	0.20	0.35	0.10	0.05
(+)-trans-Epivincaminic acid ethyl ester	0.53	0.36	0.40	0.47	0.38	0.45	0.57	0.45	0.65	0.39	0.27
(+)-trans-Vincaminic acid ethyl ester	0.68	0.49	0.54	0.56	0.58	0.58	0.74	0.68	0.74	0.61	0.39
(+)-cis-Vincamone	0.88	0.62	0.68	0.71	0.63	0.68	0.78	0.64	0.78	0.82	0.40
(+)-cis-Apovincaminic acid phenyl ester	0.92	0.68	0.72	0.78	0.68	0.75	0.82	0.62	0.78	0.86	0.40
(+)-cis-Apovincaminic acid ethyl ester	1.00	0.76	0.78	0.82	0.76	0.82	0.85	0.64	0.78	0.96	0.40
(+)-cis-Apovincamine	1.13	0.85	0.85	0.98	0.82	0.88	0.98	0.71	0.88	1.14	0.50
(+)-cis-Vincamenine	1.29	0.97	0.92	0.98	0.91	0.96	0.98	0.71	0.77	1.27	0.50
(+)-cis-Dehydrovincamine	1.40	1.05	1.00	1.25	1.15	1.25	1.50	1.23	1.25	1.52	0.60
(+)-cis-Dehydroepivincamine	1.55	1.15	1.10	1.35	1.27	1.35	1.62	1.33	1.35	1.64	0.65
(+)-cis-10-Bromovincamine	1.62	1.26	1.25	1.48	1.37	1.46	1.74	1.43	1.50	1.76	0.80
(+)-cis-11-Bromovincamine	1.77	1.39	1.37	1.62	1.51	1.62	1.89	1.57	1.63	1.93	0.88
(+)-cis-Vincaminic acid ethyl ester	2.17	1.65	1.61	1.90	1.74	1.93	2.18	1.71	1.76	2.35	1.04
(+)-cis-Vincamine	2.35	1.79	1.75	2.15	1.98	2.18	2.49	1.99	2.04	2.60	1.14
(-)-cis-Vincamine	2.35	1.79	1.75	2.15	1.98	2.18	2.49	1.99	2.04	2.60	1.14
(+)-cis-Epivincaminic acid ethyl ester	3.09	2.56	2.36	2.86	2.97	3.06	3.39	2.94	2.63	4.64	1.59
(+)-cis-10-Methoxyvincamine	3.09	2.35	2.32	2.86	2.62	2.84	3.39	2.66	2.63	3.60	1.47
(+)-cis-Epivincamine	3.43	2.79	2.61	3.24	3.26	3.35	3.92	3.24	3.08	5.34	1.73
(+)-cis-Vincanole	3.43	2.79	2.61	3.09	3.26	3.35	3.54	2.94	2.63	4.28	1.78
(+)-cis-Isovincanole	3.96	3.13	2.97	3.58	3.54	3.85	4.04	3.24	3.08	4.88	1.88

TABLE 8.15

CAPACITY RATIOS, k' , FOR EBURNANE ALKALOIDS ON μ BONDAPAK CN WITH DIFFERENT ELUENTS⁵⁵

Column, μ Bondapak CN (300x3.9 mm I.D.), flow-rate 1 ml/min, detection UV, 280 nm.
 mobile phases: A = hexane-chloroform-acetonitrile (65:20:15); B = 70:20:10;
 C = 75:20:5.

Alkaloid	Eluent		
	A	B	C
(+)-cis-Vincamone	0.11	0.27	0.38
(+)-cis-Apovincaminic acid ethyl ester	0.23	0.32	0.57
(+)-cis-Apovincamine	0.25	0.40	0.64
(+)-cis-Vincamenine	0.28	0.43	0.64
(+)-cis-Vincaminic acid ethyl ester	0.72	0.86	1.36
(+)-cis-Vincamine	1.00	1.07	1.57
(-)-cis-Vincamine	1.00	1.07	1.57
(+)-cis-Epivincaminic acid ethyl ester	1.15	1.20	1.80
(+)-cis-10-Methoxyvincamine	1.28	1.61	1.95
(+)-cis-Epivincamine	1.34	2.06	3.79
(-)-cis-Epivincamine	1.34	2.06	3.79
(+)-cis-Vincanole	1.57	2.14	3.86
(+)-cis-Isovincanole	2.62	3.00	4.57

For the analysis of reserpine and hydrochlorothiazide, Butterfield et al.³⁰ preferred a straight-phase separation. Because of the low concentration of the alkaloid compared to the thiazide drug, it was desirable to maximize the response of the former by eluting it from the column first. It was achieved by the straight-phase separation technique. On a microparticulate silica gel column, the drugs mentioned and a number of decomposition products could be analyzed (Table 8.13).

To analyze the hallucinogenic alkaloids in *Psilocybe* mushrooms White³⁷ used a polar mobile phase - methanol - water - aqueous 1 M ammonium nitrate (24:5:1) to which some ammonia was added (pH 9.7) - and a silica gel column (Fig.8.10). By increasing amounts of ammonia, the separation of psilocybin and baeocystin (demethylpsilocybin) could be improved. Similar solvent systems have been applied to the analysis of drugs of abuse - including strychnine^{19,22} (Table 7.8).

Christiansen et al.^{59,66,78} modified the method reported by White³⁷. By using a different solvent ratio, the separation of the alkaloids was improved, the k' of psilocin particularly was changed (Fig.8.11). The two-step extraction method employed guaranteed at least a 98% extraction of psilocybin. As extracting solvent, 10% 1 M ammonium nitrate in methanol was used.

Hara et al.⁴² demonstrated a systematic approach to solvent system optimization with the separation of a series of *Uncaria* and *Gardneria* alkaloids.

Because the separation of some eburnane alkaloids could not be achieved in a reversed-phase HPLC system⁵⁴, normal-phase HPLC was also investigated for the separation of these alkaloids⁵⁵. Using silica gel with a two-component mobile phase (chloroform - methanol) relatively poor separations were obtained. Addition of hexane to the mobile phase gave only a slight improvement. Four-component systems gave the best results. A hexane - chloroform - acetonitrile - methanol (55:25:20:3) eluent was found to be optimal. By choosing an appropriate mixture, difficult separations of stereo and structural isomers could be obtained (Table 8.14). Also, group separation was obtained on silica gel. A cyano-group bonded stationary phase gave simi-

lar results to silica gel (Table 8.15).

The applications of some straight-phase systems to separation problems with *Vinca* alkaloids have been shown (Fig.8.12)⁷⁵.

Whelpton⁸⁴ reported the analysis of physostigmine in plasma using a silica gel column with the mobile phase methanol - 1 M ammonium nitrate (pH 8.6)(9:1) in combination with an electrochemical detection method.

8.5. DETECTION

Verpoorte and Baerheim Svendsen⁷ determined the optimum wavelength of detection for some indole alkaloids for a fixed wavelength detector equipped for 254 and 280 nm detection.

The ratio between the absorbance at 254 and 280 nm, which is characteristic for each compound, was used for the identification of drugs of abuse - including strychnine (Table 2.2, 2.3)³⁵, the ratio 220/254 nm has also been used⁸².

Sams²⁷ oxidized reserpine prior to its HPLC-analysis. The 3-dehydro derivative formed was detected fluorimetrically (excitation 390 nm, emission 470 nm). This allowed a more specific and sensitive detection.

Ellipticine and derivatives can be detected at their UV-maximum of about 300 nm with a detection limit of about 1 ng. For some alkaloids, fluorescence detection is even more sensitive - allowing detection in pg amounts (excitation 305 nm, emission 470 nm). A poorly fluorescent compound such as 9-hydroxy-2-methylellipticinium acetate could be transformed to its fluorescent acetoxy derivative³⁶. Bykadi et al.⁷⁰ also detected ellipticine fluorimetrically (excitation 360 nm, emission 455 nm).

Sasse et al.⁴⁵ used the difference in fluorescence maxima of harmane alkaloids in the quantitation of the alkaloids after their separation (Fig.8.4). Fluorimetric detection with a detection limit of about 10 pg was about 100 times more sensitive than UV detection.

Fluorescence detection has also been applied in the analysis of *Psilocybe* alkaloids^{46,59} (Fig.8.11). The minimum detectable amount was for fluorimetric detection 250 pg (excitation 267 nm, emission 335 nm) and for UV detection 30 ng (267 nm) for psilocybin, for psilocin the detection limits were 7 ng (excitation 260 nm, emission 312) and 150 ng (267 nm). Psilocin - a phenolic compound - can also be detected with an electrochemical detector³⁹.

Lang et al.⁸⁰ reported the use of a post-column air-segmented reactor to enable fluorimetric detection of reserpine. As oxidizing reagent: sulfuric acid - sodium nitrite was used, which converted reserpine into 3,4-dehydroreserpine. The reaction product was detected by measuring emission above 470 nm after excitation at a wavelength of 395 nm.

For the detection of N-propylajmaline, fluorimetric detection was employed (excitation 242 nm, emission 320 nm)⁸³.

Whelpton⁸⁴ detected physostigmine electrochemically, using an oxidation potential of 0.8 V vs SSCE. The capacitance conductivity detector as described by Hashimoto et al.³² was tested on a series of alkaloids - including some indole alkaloids.

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TABLE 8.16

INDOLE ALKALOIDS IN THE CONTEXT OF HPLC ANALYSIS OF DRUGS OF ABUSE (CHAPTER 7 and 9)

Alkaloids	Ref.	Ref. in Chapter 7	Ref. in Chapter 9
S*	3		1
S	5	11	
S	12	15	
B	13	17	
S	15	18(Fig.7.2)	
S	19	22(Table 7.8, Fig.7.16)	
S	22	32	
S	24	38	
S	35	56	
Psilocin	39	61	
S	48	79	
S	51	91(Table 7.11)	
S	52	93(Fig.7.6)	
S	63,64,65	98,99,100	
S	72	113	
S	82	121(Table 7.6)	

* Abbreviations used in Tables 8.16 - 8.20

Ajmal	Ajmaline	RSC	Rescinnamine
Alst	Alstonine	RSP	Reserpine
B	Brucine	S	Strychnine
α -C	α -Colubrine	Sarp	Sarpagine
β -C	β -Colubrine	THalst	Tetrahydroalstonine
D	Diaboline	V	Vomicine
I	Icajine	VCR	Vincristine
4OHS	4-hydroxystrychnine	VLB	Vinblastine
Phys	Physostigmine	Yoh	Yohimbine
PSS	Pseudostrychnine		

TABLE 8.17

HPLC ANALYSIS VARIOUS COMPOUNDS INCLUDING INDOLE ALKALOIDS

ALKALOIDS *	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID (mm)	MOBILE PHASE	REF.
S,B,xanthines		Dynamic coating HPLC	Corasil I or II, dynamically coated with 1.1% Poly G-300	500x1	Heptane-EtOH(10:1) sat. with stationary phase	1,2
S,B,RSP,yoh,Thalst, ajmalicine,serpentine,alst,16 others		Analysis alkaloids	Merckosorb Si60 5 µm	300x2	CHCl ₃ -MeOH(9:1),(8:2),(7:3) Et ₂ O ³ -MeOH(8:2),(7:3),(6:4)	7
S,opium alkaloids, quinine,cinchonine, nicotine,atropine, cocaine		Separation on ion-exchange resins (ligand-exchange LC)	Hydrolyzed Poragel PT loaded with Cu ⁺⁺ Bio-Rad PC20 loaded with Cu ⁺⁺	470x6.3 470x6.3	0.06M NH ₄ OH in 33% EtOH 0.2 M NH ₄ OH in 33% EtOH 0.05M NH ₄ OH in 33% EtOH 0.03M NH ₄ OH in 33% EtOH	9,16,21
S,B,RSP,yoh,Cinchona alkaloids,scopolamine, emetine,caffeine		Detection with conductance detector	Silica gel 10 µm		CHCl ₃ -MeOH-hexane(7:3:10)	32
B,codeine,narceine, cinchonidine,colchicine,aconitine,caffeine	Santonine	Effect solvent composition on retention	Lichrosorb RP2 10 µm	120x3.5	MeOH-H ₂ O(1:4),(2:3),(3:2),(4:1) MeOH	40
S,RSP,yoh,opium alkaloids,cinchonine,quinine,atropine,ephedrine,acridine		Separation on styrene-divinylbenzene copolymer (Table 8.4, Fig.8.6)	Hitachi gel 3010 10 µm	220x4.6	ACN-0.02M NH ₄ OH(6:4) ACN-0.02M tetrabutylammonium-hydroxide(3:7),(6:4)	43
Ajmaline,various others	Various drugs	Separation basic drugs with non-aqueous ionic solvents	Spherisorb S5W silica	250x4.9	MeOH-hexane(85:15) containing 0.02% HClO ₄	79

TABLE 8.18

HPLC ANALYSIS INDOLE ALKALOIDS IN PLANT MATERIAL AND IN NATURALLY OCCURRING MIXTURES

ALKALOIDS *	AIMS	STATIONARY PHASE	COLUMN DIM. LxID (mm)	MOBILE PHASE	REF
Speciophylline,uncarine F, isopteropodine,mitraphylline, isomitraphylline,rhynchophylline,	Separation <i>Mitragyna</i> alkaloids	Corasil C10	500x1.7	MeOH-H ₂ O(4:1)	

*For abbreviations used see footnote Table 8.16

isorhynchophylline, rotundifoline isorotundifoline, rhynchociline, ciliaphylline					4
S, B, serpentine, alst	Separation (Fig. 8.9)	Merckosorb Si60 5 μm	300x2	Et ₂ O-MeOH-DEA(90:10:1), (70:30:1)	8
<i>Tabernaemontana</i> alkaloids	Semipreparative separation	Porasil C Porasil B C18	610x9.5 610x9.5	CHCl ₃ -MeOH-NH ₄ OH(99:0.8:0.2) MeOH	
		Al ₂ O ₃	610x9.5	MeOH-H ₂ O-NH ₄ OH(90:5:5) CHCl ₃ -hexane(9:1)	17
S, B, α -C, β -C, D, 40HS, PsS, I, V, spermoS, alst, serpentine	Separation <i>Strychnos</i> alkaloids (Table 8.12)	Merckosorb Si60 5 μm	300x2	Et ₂ O-DEA(99:1) Et ₂ O-MeOH(1:1)	18
<i>Rauwolfia</i> alkaloids	Analysis in plant material	No details available			23
26 <i>Catharanthus</i> alkaloids	Quality control (Table 8.2, Fig. 8.2)	Lichrosorb RP8	250x4	ACN-0.01M aq. (NH ₄) ₂ CO ₃ (47:53)	26
Ajmalicine	Analysis in plant material	μ Porasil	300x4	n-hexane-(isopr) ₂ O-MeOH(90:10:3)	28, 34
Bisnordihydrotoxiferine, bisnor-C-alkaloid H, caracurine V	Isolation from <i>Strychnos</i> species	μ Porasil	300x3.9	EtOAc-MeOH-25% NH ₄ OH(90:10:1), (80:25:2)	33, 41, 44
Ellipticine and derivatives	Separation (Table 8.5, 8.6)	μ Bondapak C18	300x4	MeOH-0.005M heptanesulfonic acid, 0.032M AcOH(7:3), (3:1) MeOH-0.005M pentanesulfonic acid, 0.032M AcOH(7:3) MeOH-0.02M NH ₄ OAc(3:1)	36
Psilocybin, psilocin, baeocystin	Analysis <i>Psilocybe</i> mushrooms (Fig. 8.10)	Partisil 5, 6 μm	250x4.6	MeOH-H ₂ O-1M NH ₄ NO ₃ (24:5:1)(pH 9.7)	37
RSP, yoh, <i>Uncaria</i> and <i>Gardneria</i> alkaloids	Solvent system optimization procedure	Spherosil XOA-600, 5 μm or Wakogel LCH-10, 10 μm	100x0.5	n-hexane-THF, CH ₂ Cl ₂ -DEA, CHCl ₃ -MeOH, THF-MeOH in various ratios Stepgradient CH ₂ Cl ₂ -MeOH-DEA(100:0.1:0.01) to (100:1:0.01)	42
Harmol, harmine, harmalol, harmaline	Analysis in tissue culture of <i>Peganum harmala</i> (Fig. 8.4)	Lichrosorb RP8 7 μm	250x3	MeOH-H ₂ O-HCOOH(166:34:1) buffered at pH 8.5 with TrEA	45
Psilocybin, psilocin	Analysis <i>Psilocybe</i> mushrooms (Fig. 8.1)	Partisil SCX-10	250x4.6	MeOH-H ₂ O(1:4) containing 0.2% NH ₄ -phosphate and 0.1% KCl, pH 4.5	46
Psilocybin, psilocin	Analysis <i>Psilocybe</i> mushrooms	μ Bondapak C18	300x4	MeOH-0.4% phosphate buffer(pH 7.2)-cetrimonium bromide(40:60:0.15)	49
<i>Catharanthus</i> alkaloids	Isolation from tissue culture	Lichrosorb RP18		MeOH-H ₂ O-TrEA(75:25:0.1), (95:5:0.5)	50
22 Eburnane alkaloids	Separation eburnane alkaloids (Table 8.3, Fig. 8.3)	μ Bondapak C18 or Lichrosorb RP18	300x3.9 250x4.6	ACN-0.01M (NH ₄) ₂ CO ₃ (2:3), (1:1), (3:2), (7:3), (4:1)	54

21 Eburnane alkaloids	Separation eburnane alkaloids (Table 8.14, 8.15)	Micropak Si 10 or Lichrosorb Si60 5 μ m μ Bondapak CN	250x2 250x4.6 300x3.9	Hexane-CHCl ₃ -ACN-MeOH(55:25:20:3) and various other ratios Hexane-CHCl ₃ -ACN(65:20:15),(7:2:1) (75:20:5)	55
Psilocybin,psilocin,baeocystin	Analysis <i>Psilocybe</i> mushrooms	μ Bondapak C18	300x3.9	0.05M heptanesulfonic acid in H ₂ O-MeOH(3:1) or H ₂ O-ACN(3:1),pH 3.5 w/ft AcOH 0.05M tetrabutylammonium in H ₂ O-MeOH (3:1) pH 7.5 with H ₃ PO ₄	56
Tabersonine,(-) and (+)-vincadifformine,vincamine,apo- and ethylapo-vincamine,quebrachamine,N-methylquebrachamine, (-)-vincadine	Separation with inclusion LC	β -Cyclodextrin polymer	280x16	pH 5 citrate buffer	57
Psilocybin,psilocin	Analysis <i>Psilocybe</i> mushrooms (Fig.8.11)	Partisil 5, 6 μ m	250x4.6	MeOH-H ₂ O-1M aq. NH ₄ NO ₃ (pH 9.6) (22:7:1)	59,66
VCR,VLB,vindoline,vindolinine, catharanthine,leurosine,coronaridine	Separation <i>Vinca rosea</i> alkaloids	RSil-C18-HL-D 10 μ m	250x4.6	gradient MeOH-H ₂ O(1:1) to (85:15) containing 0.1%ethanolamine	60
17 Eburnane alkaloids	Separation eburnane alkaloids (Table 8.8, 8.9)	μ Bondapak CN	300x3.9	Hexane-CHCl ₃ -ACN-di-(2-ethylhexyl)-phosphoric acid in various ratios Hexane-isoprOH-DEA-camphorsulfonic acid in various ratios	73
Vincamine,vincine,vincanole, isovincanole,vincamone,vincamine	Analysis in <i>Vinca</i> extracts (Fig.8.12)	Lichrosorb Si60 5 μ m Micropak Si 10 μ Bondapak C18	250x4.6 250x2 300x3.9	Hexane-CHCl ₃ -MeOH(8:1:1) CHCl ₃ -EtOH(95:5) ACN-0.01M (NH ₄) ₂ CO ₃ (3:2)	75
18 Eburnane alkaloids	Separation stereoisomers (Table 8.10, 8.11, Fig.8.8)	Nucleosil 5CN or Nucleosil 10CN	140x4.6 250x4.6	Hexane-CHCl ₃ -MeOH, hexane-CHCl ₃ -EtOH or hexane-CHCl ₃ -isoprOH (80:18:2), (70:27:3) or (60:36:4) containing 0.002M camphorsulfonic acid, 0.001M DEA	76
9 Heteroyohimbine and 8 oxindole alkaloids	Separation on reversed phase HPLC (Table 8.1)	Spherisorb ODS 5 μ m	250x4	MeOH-H ₂ O(4:1) MeOH-H ₂ O-conc. NH ₄ OH(80:20:1) ACN-1% (NH ₄) ₂ CO ₃ (3:2) MeOH-1%(NH ₄) ₂ CO ₃ (4:1)	77
Psilocybin,psilocin,baeocystin	Analysis <i>Psilocybe</i> mushrooms	Partisil 5, 6 μ m	250x4.6	MeOH-H ₂ O-1M NH ₄ NO ₃ (pH 9.6)(22:7:1) MeOH-H ₂ O-1M NH ₄ OAc(pH 9.6)(24:5:1)	78

TABLE 8.19

HPLC ANALYSIS INDOLE ALKALOIDS IN PHARMACEUTICAL PREPARATIONS

ALKALOIDS*	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
RSP,RSC,sarp,yoh, ajmal		Analysis <i>Rauwolfia</i> alkaloids	ION-X-SC	1000x2.6	0.02M (NH ₄) ₂ HPO ₄ in MeOH-H ₂ O(3:7) pH 7.0 0.05M (NH ₄) ₂ HPO ₄ in MeOH-H ₂ O(3:7) pH 8.0 gradient from 0.01M, pH 7.0 to 0.05M pH 8.0	6
RSP	Chlorothiazide, phenantrene	Analysis in pharmaceutical preparations	Corasil C18	1220x2.3	MeOH-0.5% NH ₄ Cl(55:45)(pH 5.6)	11
RSP	Chlorothiazide, triamterene,hy- drochlorothiazide, hydralazine,gua- nethidine,methyl- dopa	Analysis diuretic-antihyper- tensive drugs	Corasil C18 or Phenyl	1220x2.3	ACN-0.1% NH ₄ OAc(4:1),(3:2),(2:3),(1:1) MeOH-1% NH ₄ OAc(1:1) ACN-1% NH ₄ Cl(4:1),(1:1) MeOH-0.5% NH ₄ Cl(1:1) ACN-0.2% (NH ₄) ₂ CO ₃ (1:1) MeOH-0.2%(NH ₄) ₂ CO ₃ (1:1) MeOH-1% (NH ₄) ₂ CO ₃ (1:1)	20
S,12 others	Various drugs	Analysis pharmaceuticals (Table 2.5)	Partisil 10 µm	250x4.6	CH ₂ Cl ₂ -MeOH(1:3) with 1% NH ₄ OH	25
Ajmalicine	Acetanilide	Analysis in pharmaceuticals and plant material	µPorasil	300x4	n-Hexane-(isopr) ₂ O-MeOH(90:10:3)	28
RSP,RSC,degrada- tion products	Diuretics	Analysis in tablets (Table 8.13)	Lichrosorb Si60 5 µm	250x2.1	n-Hexane-isoprOH-CHCl ₃ -DEA (77:18: 5:0.01)	30
Vindesine,dehydro- vindesine,vindesine N-oxide	Phenantrene	Analysis bulk drug	µBondapak C18	300x3.9	MeOH-H ₂ O-DEA(1000:600:3),(835: 600:3) ²	31
S,various others	Various drugs	Identification in pharmaceu- ticals (Fig. 7.14)	Partisil PXS 5/25	250x4.6	Et ₂ O sat. with 50-100% H ₂ O + 0.05-0.8% DEA	38
Phys,rubreserine, pilocarpine	Methyl-,ethyl-, propyl-p-hydro- xybenzoate	Determination in aqueous so- lutions (Table 8.7, Fig.8.7)	µBondapak C18	300x3.9	MeOH-0.005M heptanesulfonic acid in H ₂ O (pH 3.6)(2:3)	47
Phys	Flurazepam,ben- zylalcohol	Collaborative study of deter- mination phys in pharmaceuti- cals	µBondapak C18	300x3.9	ACN-0.05M NH ₄ OAc (1:1)	67

*For abbreviations used see footnote Table 8.16

Vinpocetine, vincamine, epivincamine, vincaminic acid, vincamone	Diazepam	Analysis in pharmaceuticals (content uniformity)	Nucleosil 10C18	250x4.6	ACN-0.01M $(\text{NH}_4)_2\text{CO}_3$ (9:1), (7:3) which contains 0.001M trioctylmethylammonium chloride	75
RSP, methylreserpate		Post-column reactor for fluorimetric detection	Micropak Si 10 Phenyl bonded phase	250x2 not given	CHCl_3 -EtOH (95:5) ACN-0.005M NaH_2PO_4 buffer (pH 6) (7:3)	80

TABLE 8.20

HPLC ANALYSIS INDOLE ALKALOIDS IN BIOLOGICAL MATERIAL AND IN TOXICOLOGICAL ANALYSIS

ALKALOIDS*	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
S		Determination in grain baits	μ Porasil	300x4	CHCl_3 -MeOH (9:1)	14
RSP		Determination in plasma	μ Bondapak C18	300x4	MeOH-0.01M aq. heptanesulfonate (65:35)	27
S		Determination in grain baits and stomach contents	μ Bondapak C18	300x4	MeOH-0.005M KH_2PO_4 buffer (pH 3) (3:2)	29
Ellipticine and derivatives		Analysis in biological fluids (Table 8.5, 8.6)	μ Bondapak C18	300x4	MeOH- H_2O (7:3), idem containing 0.005M heptane- or pentanesulfonate, and 0.032M AcOH MeOH- H_2O (3:1) containing 0.005M heptanesulfonate and 0.032M AcOH MeOH- H_2O (3:1) containing 0.02M NH_4OAc	36
Vincamine, desoxy-vincaminamide		Analysis in plasma	Spherisorb ODS 5 μm	150x4.6	ACN-0.02M phosphate buffer (pH 2.3) (1:1)	53
Vincamine, 14-epi-, and apo-vincamine, papaverine		Determination in biological fluids	μ Bondapak C18	300x3.9	MeOH-0.01M $(\text{NH}_4)_2\text{CO}_3$ (3:1)	58
Alcuronium, tubocurarine		Determination in biological fluids	μ Bondapak C18	300x6.4	MeOH- H_2O (4:1) containing 0.25% AcOH and 0.005M Na-dodecylsulfate	61
S, yoh, cocaine, papaverine, heroin	Various drugs	Determination papaverine in blood	Micropak CN 10	300x4	n-Hexane- CH_2Cl_2 -ACN-propylamine (50:25:25:0:1) ²	62
11-bromovincamine, vincamine		Determination in plasma	μ Bondapak C18	300x4	ACN-0.1% NaH_2PO_4 (35:65) (pH 3.5)	69
Ellipticine, 9-hydroxyellipticine, 11-demethylellipticine		Analysis in biological samples	μ Bondapak C18	300x4	ACN-0.01M NaH_2PO_4 (36:64), (25:75) pH 3.5	70

*For abbreviations used see footnote Table 8.16

S,quinine	Determination S in biological fluids	Silica gel 10 μm	250x2.6	MeOH-conc. NH_4OH (99.25:0.75)	71
Vinpocetine,vincaminic acid,apovincaminic acid	Determination in plasma	Lichrosorb RP8 10 μm	200x4.6	ACN-0.0075M phosphate buffer (pH 3.5)(28:72)	74
Vinpocetine,apovincamine	Determination in plasma	Lichrosorb RP8	250x4.6	ACN-0.01M $(\text{NH}_4)_2\text{CO}_3$	75
S,B	Determination in urine and tissue extracts	Lichrosorb Si60 7 μm	250x4.6	MeOH- H_2O -conc. NH_4OH (85:14.2:0.8)	81
N-propylajmaline	Determination in plasma	Lichrosorb Si100 5 μm coated with 0.2M HClO_4 and 0.8M NaClO_4	150x4.5	n-BuOH-1,2dichloroethane-hexane (15:40:45)	83
Phys,rubreserine, eseroline	Determination in plasma	Spherisorb 5 μm	250x4.5	MeOH-1M NH_4NO_3 (pH 8.6)(9:1)	84

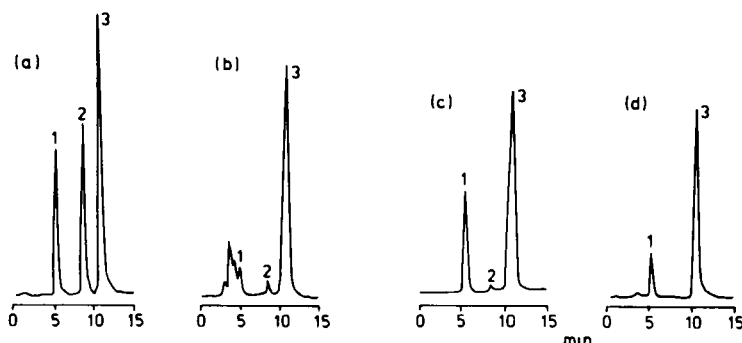


Fig. 8.1. Separation of *Psilocybe* alkaloids⁴⁶

Column Partisil SCX-10 (250x4.6 mm ID), protected with a precolumn (30x2.8 mm ID) packed with 30 μ m pellicular beads, mobile phase methanol - water (1:4) containing 0.2% ammonium phosphate and 0.1% potassium chloride (pH 4.5), flow rate 1 ml/min, temperature 50°C. Peaks: 1, psilocybin; 2, psilocin; 3, dimethyltryptamine (internal standard). Chromatogram a: standard mixture, detection UV 267 nm; chromatogram b: mushroom extract, detection UV 267 nm; chromatogram c: standard mixture, fluorescence detection (excitation 267 nm, emission 335 nm); chromatogram d: mushroom extract, fluorescence detection.

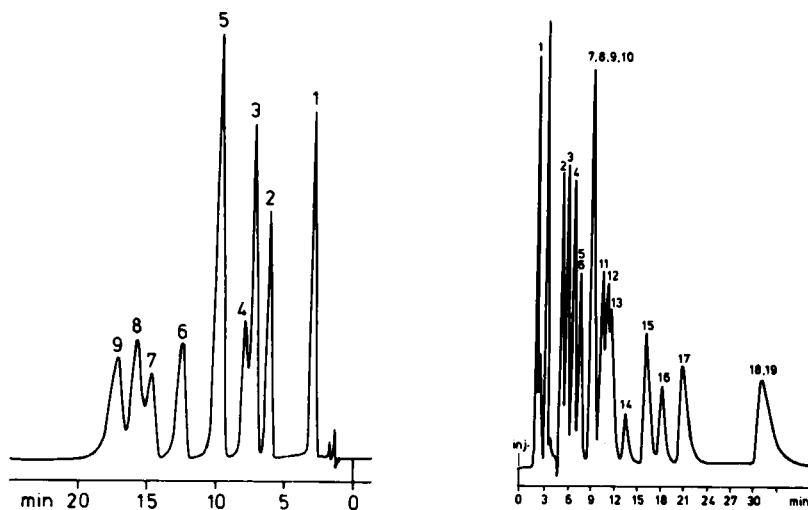


Fig. 8.2. Separation of some *Catharanthus* alkaloids²⁶

Column Lichrosorb RP8 (250x4 mm ID), mobile phase acetonitrile - 0.01 M ammonium carbonate (47:53), flow rate 1.5 ml/min, detection UV 298 nm. Peaks: 1, lochnerine; 2, vindoline; 3, vincristine; 4, ajmalicine; 5, catharanthine; 6, vinblastine; 7, tetrahydroalstonine; 8, leurosine; 9, desacetoxyvinblastine.

Fig. 8.3. Separation of eburnane alkaloids on an octadecyl column⁵⁴

Column μ Bondapak C18 (300x3.9 mm ID), mobile phase acetonitrile - 0.01 M ammonium carbonate (6:4), flow rate 1 ml/min, detection UV 280 nm. Peaks: 1, (+)-cis-apovincamine; 2, (+)-cis-dehydroepivincamine; 3, (+)-cis-dehydrovincamine; 4, (+)-trans-vincaminic acid ethyl ester; 5, (+)-cis-epivincamine; 6, (-)-cis-epivincamine; 7, (+)-cis-vincamine; 8, (-)-cis-vincamine; 9, (+)-cis-epivincaminic acid ethyl ester; 10, (+)-trans-epivincaminic acid ethyl ester; 11, (+)-cis-vincamone; 12, (+)-cis-vincanole; 13, (+)-cis-vincaminic acid ethyl ester; 14, (+)-cis-isovincanole; 15, (+)-cis-apovincamine; 16, (+)-trans-apovincaminic acid ethyl ester; 17, (+)-cis-apovincaminic acid ethyl ester; 18, (+)-cis-apovincaminic acid phenyl ester; 19, (+)-cis-vincamenine.

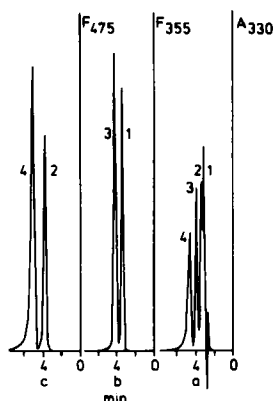


Fig. 8.4. Separation of harmaline alkaloids⁴⁵

Column Lichrosorb RP8 7 μ m (250x3 mm ID), mobile phase methanol - water - formic acid (166:34:1) buffered with triethylamine at pH 8.5, flow rate 1 ml/min, detection UV 330 nm (a), fluorescence (excitation 304 nm, emission 355 nm)(b) and fluorescence (excitation 396 nm, emission 475 nm)(c). Peaks: 1, harmol; 2, harmalol; 3, harmine; 4, harmaline.

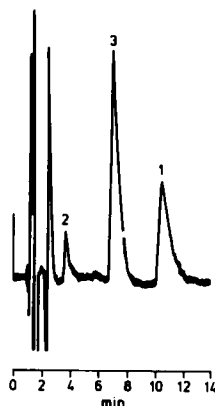


Fig. 8.5. Separation of ellipticine and related alkaloids, isolated from mouse blood⁷⁰
Column μ Bondapak C18 (300x4.0 mm ID), protected with a precolumn (50x4.6 mm ID) packed with a 40 μ m pellicular material, mobile phase acetonitrile - 0.01 M sodium dihydrogen phosphate (1:3)(pH 3.5 with 2 N phosphoric acid), flow rate 1.4 ml/min, detection UV 300 nm. Peaks: 1, ellipticine; 2, 9-hydroxyellipticine; 3, 11-demethylellipticine (internal standard).

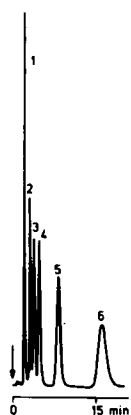


Fig. 8.6. Separation of some alkaloids on a porous polymer⁴³

Column Hitachi Gel 3010 (macroporous styrene-divinylbenzene copolymer), 10 μ m (220x4.6 mm ID), mobile phase acetonitrile - water (6:4) containing 0.02 M ammonia, flow rate 2 ml/min, detection UV 254 nm. (see also Table 8.4). Peaks: 1, morphine; 2, codeine; 3, papaverine; 4, yohimbine; 5, noscapine; 6, reserpine. (reproduced with permission from ref. 43, by courtesy of the American Chemical Society)

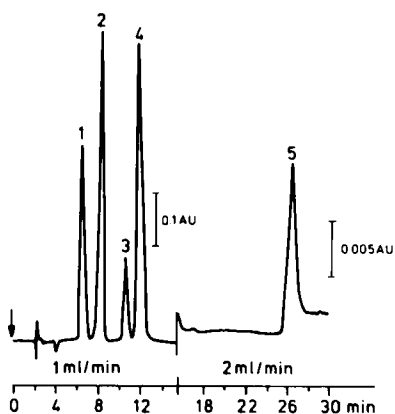


Fig. 8.7. HPLC analysis pilocarpine, physostigmine, its degradation products and preservatives in pharmaceutical preparations⁴⁷

Column μ Bondapak C18 (300x3.9 mm ID), mobile phase methanol - water (2:3) containing 0.005 M heptanesulfonic acid (pH 3.6), flow rate 1 ml/min, detection UV 235 nm and 292 nm. Peaks: 1, pilocarpine; 2, salicylate; 3, methyl-p-hydroxybenzoate; 4, physostigmine; 5, propyl-p-hydroxybenzoate. (see also Table 8.7)

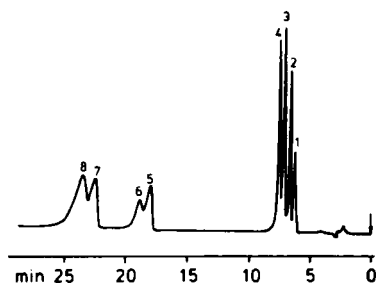


Fig. 8.8. Separation of eight optical isomers of vincamine⁷⁶
 Column Nucleosil 5CN (150x4.6 mm ID), mobile phase hexane - chloroform - ethanol (70:27:3) containing 0.002 M (+)-10-camphorsulfonic acid and 0.001 M diethylamine, flow rate 1 ml/min, detection UV 280 nm. Peaks: 1, (+)-cis-epivincamine; 2, (-)-cis-epivincamine; 3, (+)-cis-vincamine; 4, (-)-cis-vincamine; 5, (+)-trans-epivincamine; 6, (-)-trans-epivincamine; 7, (+)-trans-vincamine; 8, (-)-trans-vincamine.

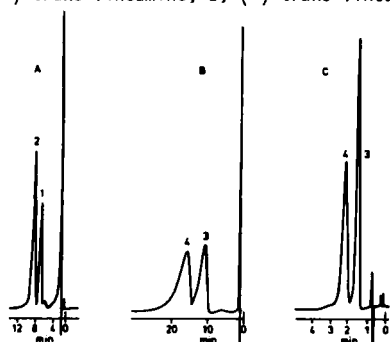


Fig. 8.9. Separation of *Strychnos* alkaloids^{8,18}
 Column Merckosorb Si60 5 μ m (300x2 mm ID), mobile phase A: diethyl ether - methanol - diethylamine (70:30:1), flow rate 1 ml/min; B: diethyl ether - methanol (1:1), flow rate 1.15 ml/min; C: diethyl ether - methanol - diethylamine (90:10:1), flow rate 1.36 ml/min. Detection UV 254 nm. Peaks: 1, alstonine; 2, serpentine; 3, strychnine; 4, brucine.



Fig. 8.10. Separation of *Psilocybe* alkaloids³⁷
 Column Partisil 5, 6 μ m (250x4.6 mm ID), mobile phase methanol - water - 1 M ammonium nitrate (24:5:1) buffered at pH 9.7 with ammonia, flow rate 2 ml/min, detection UV 254 nm. Peaks: 1, psilocin; 2, psilocybin; 3, baeocystin (in mushroom extract).

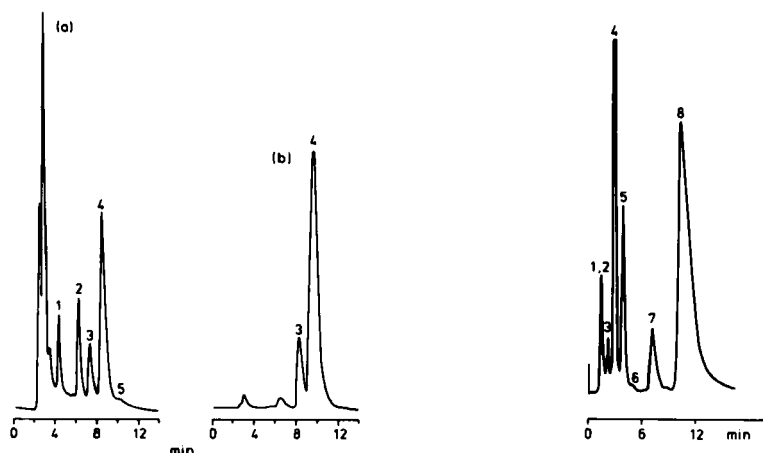


Fig. 8.11. Separation of *Psilocybe* alkaloids⁵⁹
 Column Partisil 5, 6 μm (250x4.6 mm ID), mobile phase methanol - water - 1 M ammonium nitrate (22:7:1) buffered to pH 9.6 with ammonia, flow rate 1 ml/min, detection UV 254 nm (a), fluorescence (excitation 267 nm, emission 335 nm) (b). Peaks: 1, 2 and 3, unknown; 4, psilocybin; 5, psilocin.

Fig. 8.12. Separation of vincanole and isovincanole in production mother liquor⁷⁵
 Column Micropak Si-10 (250x2 mm ID), mobile phase chloroform - ethanol (95:5), flow rate 20 ml/h, detection UV 280 nm. Peaks: 1, toluene; 2, unknown; 3, vincamone; 4, vincamenine; 5, unknown; 6, unknown; 7, isovincanole.

Chapter 9

ERGOT ALKALOIDS

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The ergot alkaloids can be divided into two main groups: the lysergic acid alkaloids and the clavine alkaloids. The analytical problems concerning the lysergic acid alkaloids can be summarized as follows:

1. Analysis of LSD and related compounds.
2. Separation of ergometrine and the ergotamine and the ergotoxine groups of alkaloids.
3. Separation of the individual alkaloids within each of the groups mentioned.
4. Separation of the C-8 stereoisomers (-ine/inine, normal/iso-).
5. Separation of the dihydroderivatives of the ergotoxine group of alkaloids and ergotamine.

For each of the separations mentioned, HPLC has been used.

The analysis of LSD is sometimes described in connection with the analysis of drugs of abuse^{5,9,20,32}. Reviews on HPLC analysis of LSD have been given^{16,49,59}. Lurie and Weber^{32,49} described a semipreparative HPLC separation of LSD to enable further identification of it by means of different spectral data. Twitchett et al.²⁸ reported the analysis of LSD in body fluids by means of a combination of HPLC, fluorimetry and radioimmuno-assay.

The separation of the naturally occurring ergot alkaloids derived from lysergic acid in groups has been investigated in a long series of studies^{19,25,34,35,36,41,43,50,52,57,58,60,61} and so has the separation of the various components within each group, particularly within the ergotoxine group^{25,34,36,41,43,51,60,61}. The C-8 stereoisomers can usually be separated without difficulty^{10,14,17,19,25,26,34,36,41,43,47,51,52,60,61}; the separation of the dihydroderivatives of the ergotoxine group of alkaloids has also been achieved^{6,11,19,25,33,34,41,60,61}. Stability studies of ergotamine¹⁷, ergometrine³¹ and dihydroergotamine⁴⁷ have been performed using HPLC for the separation of the alkaloid and its degradation products. Schauwecker et al.²² developed a method for the trace enrichment of ppb quantities of ergot alkaloids in urine.

HPLC analysis of clavine alkaloids has been carried out by Wurst et al.²⁹ and Eckers et al.^{57,58}.

9.1. REVERSED-PHASE HPLC

Reversed-phase HPLC has been widely used for the analysis of both LSD and ergot alkaloids. The first reversed-phase separation of ergot alkaloids was reported by Jane and Wheals³ in connection with the analysis of LSD. Pellicular beads with chemically bonded octadecyl groups were used in combination with methanol - 0.1% aqueous ammonium carbonate (3:2) as mobile phase. However, the mobile phase proposed by Vivilecchia et al.⁶ for the separation of the dihydroergotoxine alkaloids on octadecyl columns (acetonitrile - aqueous ammonium carbonate) has been

TABLE 9.1

CAPACITY FACTORS (k') AND SEPARATION FACTORS FOR ERGOT ALKALOIDS ON REVERSED-PHASE PACKINGS^{25,34}Column: Lichrosorb RP2 (5 μ m), RP8 (10 μ m) or RP18 (10 μ m) (250x2 or 4 mm ID), mobile phase acetonitrile - 0.01M aqueous ammonium carbonate (2:3), detection UV 320 or 280 nm.

Alkaloids	Lichrosorb RP2		Lichrosorb RP8		Lichrosorb RP18	
	k'	α	k'	α	k'	α
Lysergic acid	0.16		0.20		0.10	
Ergometrine maleate	1.54		1.11		0.81	
Ergometrine	2.27	1.47	2.34	2.11	1.84	2.27
Ergotamine tartrate	5.34	2.35	7.51	3.21	7.27	3.95
Ergocornine	6.24	1.17	11.00	1.46	11.86	1.63
Ergocryptine	7.8	1.25	15.14	1.37	18.65	1.57
Ergocristine	8.45		16.66	1.10	20.50	1.10
Ergotamine	9.87	1.08	-	1.75	29.6	
Ergocornine	12.91	1.53	29.17		38.2	1.86
Ergocryptinine	14.50	1.12	43.06	1.47	60.6	1.58
Dihydroergotamine tartrate	-		-		7.94	
Dihydroergocornine methanesulfonate	7.15		9.43		9.7	
Dihydroergocryptine methanesulfonate	9.11	1.27	13.60	1.44	14.6	1.50
Dihydroergocristine methanesulfonate	9.82	1.08	14.91	1.10	17.60	1.20

TABLE 9.2

SEPARATION OF ERGOTAMINE AND ITS DECOMPOSITION PRODUCTS¹⁷Relative capacity factors ($k'_r = k'_I/k'_E$) of decomposition product I (relative to ergotamine E)Column μ Bondapak C18 (300x4 mm ID), mobile phase acetonitrile - 0.01M aqueous ammonium carbonate, linear gradient in 15 min from 3:37 to 1:1, flow rate 8.0 ml/min, detection UV 320 nm.

Alkaloid	k'_r	Rel. standard deviation (%)
Ergotamine	1.18	0.3
Aci-ergotamine	0.77	0.4
Aci-ergotamine	0.98	0.3
Lysergic acid amide	0.45	1.0
Isolysergic acid amide	0.61	0.8
Lysergic acid	0.08	1.2
Isolysergic acid	0.18	1.5

TABLE 9.3

SEPERATION OF SOME ERGOT ALKALOIDS³⁶Column Lichrosorb RP18, 10 μ m (250x4 mm ID), mobile phase 0.1% ammonium acetate in acetonitrile - water (35:65), flow rate 3.5 ml/min, detection UV 254 nm.

Alkaloids	Retention time(min)
Ergometrine	1.6
Ergometrine	2.4
Papaverine(internal standard)	4.0
Ergosine	6.2
Ergotamine	6.6
Ergocornine	10.0
Ergocryptine	11.4
Ergocristine	16.2
Ergotamine	27.0
Ergocornine	42.0
Ergocryptinine	48.0
Ergocristinine	56.0

TABLE 9.4

SEPARATION OF ERGOTOXINES AND DIHYDROERGOTOXINES⁶⁰

Alkaloid	k'
Ergocornine	4.22
Dihydroergocornine	4.38
α -Ergocryptine	5.00
Dihydro- α -ergocryptine	5.44
β -Ergocryptine	5.78
Dihydro- β -ergocryptine	5.91

Column Lichrosorb RP18 10 μ m (250x4.6 mm ID), mobile phase tetrahydrofuran - 0.01 M aqueous ammonium acetate (2:3), flow rate 1.5 ml/min, detection UV 322 nm (Fig.9.5).

found to have wider applications for the separation of ergot alkaloids. The solvent was tested by Dolinar¹⁹ and Szepesy et al.^{25,34} in combination with different reversed-phase packings. Dolinar found that the packing with chemically bonded octyl groups was the most suitable stationary phase for the separation of ergot alkaloids with a wide range of polarities (Fig.9.1). The separations obtained on an octadecyl column were similar (Fig.9.2). On a dimethylsilyl column (RP2) and a spherical octadecyl column (Spherisorb ODS), the less polar alkaloids were not as well separated as on the above-mentioned columns; however, the more polar alkaloids were better separated (Fig.9.3).

Szepesy et al.^{25,34} investigated the effect of the chain length of the chemically bonded alkyl groups on the separation of the alkaloids. Best results for the ergotoxine and dihydroergotoxine groups of alkaloids were obtained with the octadecyl stationary phase. An increase of chain length of the alkanes caused an increase of the capacity factors of the alkaloids and, to some extent, also an increase in the selectivity (Table 9.1). The four alkaloids of the ergotoxine group, as well as three dihydroergotoxine alkaloids, could be separated. Similar systems have also been used in other investigations^{17,18,21,22,37,39,50}.

For the quantitative analysis of ergotamine and its decomposition products (Table 9.2), Bethke et al.¹⁷ found that a linear gradient was necessary when acetonitrile - aqueous ammonium carbonate was used as mobile phase. A stepwise gradient as used by Erni and Frei¹⁸ could be used, but some problems arose with the quantitation of the chromatogram. However, the stepwise gradient procedure offered possibilities for the analysis of very low concentrations of dihydroergotoxine alkaloids (ppb range). Injection volumes of up to several hundred millilitres could be applied: the alkaloids were adsorbed on the top of the column and they were subsequently eluted by means of the stepwise gradient^{18,22}.

Wehrli et al.²⁴ investigated the influence of organic bases (primary, secondary, tertiary and quaternary amines) on the stability of chemically bonded reversed-phase materials. It was found that with sodium hydroxide and quaternary amines, the silicate structure of the packing was rapidly attacked, making columns useless within 1-3 days. Decomposition of such materials is mainly due to dissolution of the silica gel. However, when working with high pH values, primary, secondary and tertiary alkylamines can be used without a noticeable decrease in the quality of the column. Triethylamine was found to be a good choice. Increase in the water content of the eluents tested (acetonitrile - water - base) gave an increase in the dissolution of the silica gel. For the analysis of ergot alkaloids, acetonitrile was preferred above methanol because of a better selectivity, a higher plate number, and a lower pressure drop. The various amines tested were compared for their influence on the retention behaviour of the

TABLE 9.5

SEPARATION OF ERGOT ALKALOIDS AND LSD²⁸

S1: column Spherisorb 5-ODS (100x4.6 mm ID), mobile phase methanol - 0.025M disodium hydrogen phosphate (65:35)(pH 8), flow rate 1.0 ml/min.

S2: column Spherisorb S5W (150x4.6 mm ID), mobile phase methanol - aqueous ammonium nitrate (3:2), flow rate 1 ml/min.

Detection UV 280 nm or fluorescence (excitation 320 nm, emission 400 nm).

Alkaloid	S1	S2		S1	S2
D-LSD	1.00(4.9ml)	1.00(3.4ml)	Dihydroergotamine	2.48	0.81
Iso-LSD	2.04	1.28	Ergocornine	1.39	0.79
D-Lysergamide	0.38	2.76	Ergocristine	2.31	0.81
D-Lysergic acid	0.23	0.65	Ergocryptine	1.86	0.78
Lysergol	0.61	0.76	Ergocryptinine	2.08	0.72
Lumi-LSD	0.53	0.85	Ergometrine	0.26	0.79
	1.15		Ergometrinine	0.47	0.79
	1.68		Ergosine	1.22	0.78
2-Oxo-LSD	0.65	0.59	Ergosinine	1.22	0.75
D-Lysergic acid monoethylamide	0.52	0.81	Ergotamine	1.57	0.81
Dihydroergocornine	2.60	0.74	Ergothioneine	>6	>2.35
Dihydroergocristine	3.68	0.76	Methysergide	0.65	0.85
Dihydroergocryptine	2.63	0.71	Methylergometrine	0.39	0.76

ergot alkaloids.

Hartmann et al.³³ found that some reversed-phase columns showed insufficient stability against a mobile phase such as acetonitrile - aqueous ammonium carbonate. However, by replacing ammonium carbonate by a 0.33 M phosphate buffer (pH 7.5) similar results could be obtained for the separation of the dihydroergotoxine alkaloids, and the problem concerning the instability of the stationary phase was avoided. Similar results were also obtained with triethylamine. A mobile phase consisting of water - acetonitrile - triethylamine (32:8:1) and an octadecyl column gave a complete resolution of all four dihydroergotoxine alkaloids (Fig.9.4). A similar method was applied by Ali and Strittmatter³⁹. Acetonitrile - water (35:65) containing 0.1% ammonium acetate and tetrahydrofuran - 0.01 M ammonium acetate (3:2) have been used in combination with octadecyl columns to separate, respectively, various ergot alkaloids (Table 9.3)³⁶ and some ergotoxine-type alkaloids (Fig.9.5, Table 9.4)⁶⁰.

To avoid decomposition of reversed-phase column material by basic solvents, Sondack³¹ preferred acetonitrile - acetic acid - water (30:1:79) in combination with an octadecyl stationary phase to separate ergometrine and its decomposition products. Twitchett and co-workers (12,27,28,46) found that a mobile phase consisting of methanol - 0.025 M aqueous disodium hydrogen phosphate (65:35)(pH 8) in combination with an octadecyl column was better suited for the analysis of LSD than a normal-phase (Table 9.5).

In connection with the development of a post-column reactor, Gfeller et al.⁴² used a stationary phase with chemically bonded diol groups, which allowed the use of exclusively aqueous mobile phases (0.01 M, pH 3, phosphate buffer). A series of alkaloids was separated, viz. dihydroergotamine and bromocryptine.

Wurst et al.^{29,43} reported the separation of clavines and lysergic acid derivatives on a stationary phase containing alkylamine groups in combination with neutral organic solvents (Tables 9.6 and 9.7). For the clavines, the best separation - under isocratic conditions - was obtained with diethyl ether - ethanol (84:16); a gradient elution was, however, found to give better results. For the alkaloids derived from lysergic acid, diethyl ether - ethanol

TABLE 9.6

RELATIVE RETENTIONS OF CLAVINE ALKALOIDS AND SIMPLE DERIVATIVES OF LYSERGIC ACID²⁹Column Micropak NH₂, 10 µm (250x2 mm ID), flow rate 1 ml/min, detection UV 225 and 240 nm.

Mobile phase S1: diethyl ether - ethanol (84:16)
 S2: diethyl ether - ethanol (80:20)
 S3: diethyl ether - isopropanol (70:30)
 S4: diethyl ether - isopropanol (60:40)
 S5: chloroform - isopropanol (90:10)
 S6: chloroform - isopropanol (80:20)

Alkaloid	S1	S2	S3	S4	S5	S6
Paspaclovine	0.11	0.11	0.11	0.14	0.12	0.12
Isoetoclovine	0.25	0.26	0.27	0.26	0.70	0.70
Lysergene	0.38	0.39	0.46	0.45	0.58	0.63
Setoclovine	0.55	0.61	0.67	0.67	0.98	1.05
Isolysergic acid amide	0.83	0.80	0.69	0.64	0.58	0.55
Lysergene	0.88	0.87	1.20	1.14	1.24	1.30
Agroclavine	1.00	1.00	1.00	1.00	1.00	1.00
Pyroclavine	1.45	1.49	1.80	1.63	1.65	1.71
Festoclavine	2.08	1.98	2.37	2.01	1.46	1.47
Penniclavine	2.80	2.65	2.97	2.72	5.05	3.85
Lysergic acid amide	2.90	2.51	2.29	1.85	4.00	2.95
Paliclavine	3.12	2.25	1.66	1.26	3.85	2.99
Elymoclavine	3.22	2.90	3.17	2.72	4.48	3.05
Lysergol	3.50	2.99	3.48	2.83	5.80	4.30
Chanoclavine	8.00	6.03	8.56	6.33	9.83	6.03
retention volume agro-clavine (ml)	4.67	3.00	4.90	3.50	2.67	1.90

TABLE 9.7

RELATIVE RETENTIONS OF ERGOT ALKALOIDS⁴³Column Micropak NH₂, 10 µm (250x2 mm ID)(A) and Lichrosorb NH₂, 10 µm (250x2 mm ID)(B), flow rate 1 ml/min, detection UV 310 nm.

Mobile phase S1: diethyl ether - ethanol (93:7) column B
 S2: diethyl ether - ethanol (88:12) column A
 S3: diethyl ether - ethanol (84:16) column A
 S4: diethyl ether - isopropanol (60:40) column A
 S5: chloroform - isopropanol (90:10) column A

Alkaloid	S1	S2	S3	S4	S5
Ergocryptine	2.82	0.50	0.47	0.31	0.13
Ergocryptinine	1.59	0.25	0.21	0.14	0.03
Ergocornine	3.06	0.64	0.56	0.32	0.12
Ergocorninine	2.00	0.34	0.30	0.18	0.05
Ergocristine	3.76	0.89	0.78	0.45	0.15
Ergocristinine	2.35	0.45	0.38	0.26	0.04
Ergostine	7.38	1.85	1.30	0.82	0.33
Ergostinine	3.15	0.75	0.57	0.37	0.05
Ergosine	8.47	1.83	1.21	0.71	0.47
Ergosinine	3.09	0.63	0.46	0.28	0.07
Ergotamine	12.44	2.25	1.94	1.08	0.53
Ergotaminine	4.56	0.94	0.88	0.52	0.07
Ergometrine	12.82	5.12	4.16	2.55	4.93
Ergometrinine	3.82	1.05	1.00	0.57	0.98
Lysergic acid amide	-	-	2.90	1.85	4.00
Isolysergic acid amide	-	-	0.83	0.64	0.58
8-Hydroxy-ergotamine	8.88	-	-	-	-
Agroclavine	1.00	1.00	1.00	1.00	1.00
retention volume agro-clavine (ml)	1.10	6.00	4.67	3.50	2.67

(88:12) or (93:7) gave very good separation. However, in this case a gradient elution was also preferred. The components of the ergotoxine group were best separated with diethyl ether - ethanol (97.5:2.5). Harzer⁶² used a column switching technique to confirm the identity of LSD. An octyl type of stationary phase and a straight-phase silica gel column were used in combination with the solvent methanol - 0.3% potassium dihydrogen phosphate in water (pH 3)(1:1).

9.2. ION-PAIR HPLC

Fluorimetric ion-pair chromatography has been applied for ergotamine, ergotamine and dihydroergotamine in connection with the analysis of pharmaceutical preparations containing tropane alkaloids^{10,26,30,38} (Chapter 4).

Lurie described ion-pair chromatography of LSD. As pairing-ion heptanesulfonic acid (0.005 M)²⁰ or methanesulfonic acid (0.005 M)^{32,49} was used in a mobile phase of water - methanol - acetic acid (59:40:1)(pH 3.5) and a microparticulate octadecyl material as stationary phase. For semipreparative work, the concentration of the pairing-ion was increased to 0.04 M in order to reduce tailing and to eliminate peak splitting (see also Chapter 7)⁵⁴⁻⁵⁶.

Post column derivatization in connection with ion-pair chromatography was used by Lawrence et al.⁴⁸ for, e.g., ergotamine (Chapter 4).

Ali and Strittmatter³⁹ studied the separation of some dihydroergot alkaloids on reversed-phase columns (octadecyl and octyl) with acetonitrile - water mixtures containing salts (citrate, acetate or bromide) as mobile phase. Best results were obtained with a mobile phase of pH 7 in combination with an octyl column. Under such conditions the dihydroergotoxine alkaloids and dihydroergotamine could be separated with three different salt-containing solvents (Table 9.8). In acidic media the separations were inadequate. The α - and β -isomers of dihydroergocryptine could only be separated at a high pH (12.3) with acetonitrile - methanol - diethylamine (375:65:21) as mobile phase, i.e. a solvent system similar to that mentioned by Hartmann et al.³³.

A normal-phase ion-pair chromatographic method for the separation of ergot alkaloids has been reported by Szepesi et al.⁶¹. The behaviour of some alkaloids upon changes in the di-(2-ethylhexyl)phosphoric acid concentration is shown in Table 9.9. The increase of the concentration of the counter-ion showed a nearly linear relationship with the increase of the capacity factor of the ergot alkaloids. Due to the strong interaction of 10-camphorsulfonic acid and the ergot alkaloids, systems with this counter-ion were unsuitable for ergot alkaloids (Table 8.9). Under basic conditions the alkaloids could also be separated using this sort of mobile phase (Table 9.10).

9.3. STRAIGHT-PHASE HPLC

Wittwer and Kluckhohn¹ analyzed LSD and a series of ergot alkaloids on silica gel with acetonitrile - diisopropyl ether as mobile phase. A similar system was used by Perchalski et al.⁷ for the determination of ergotamine in plasma. Heacock et al.² performed some preliminary investigations on the analysis of ergot alkaloids on pellicular silica gel packings. By means of chloroform - methanol - ethyl acetate - acetic acid (60:20:50:3), reasonable results were achieved. Water-deactivated columns in combination with chloroform - methanol were also used with good results.

TABLE 9.8

CAPACITY FACTORS AND SEPARATION FACTORS (α) FOR SOME ERGOT ALKALOIDS ON A REVERSED PHASE COLUMN³⁹

Column, Lichrosorb RP8, 7 μ m (250x4.6), mobile phases, S1 acetonitrile - water - triethanolamine - citric acid (45 ml + 60 ml + 0.4 ml + 0.166 g), pH = 7.1; S2 acetonitrile - water - triethanolamine - sodium acetate (45 ml + 60 ml + 1 ml + 0.3 g), pH = 7.1, adjusted by addition of two drops acetic acid; S3 acetonitrile - water - tetradecyl trimethyl ammonium bromide (45 ml + 60 ml + 0.1 g), pH = 7.1, adjusted by addition of two drops triethanolamine; S4 acetonitrile - water - ammonium carbonate (45 ml + 60 ml + 0.04 g), pH = 8.3; S5 acetonitrile - water - diethylamine (375:625:21), pH = 12.3.

ALKALOIDS	MOBILE PHASE									
	S1		S2		S3		S4		S5	
	k'	α	k'	α	k'	α	k'	α	k'	α
dihydroergotamine	2.68	1.29	3.29	1.21	2.08	1.27	4.66	1.23		
dihydroergocornine	3.45	1.43	4.00	1.43	2.65	1.45	5.72	1.44	1.30	1.35
dihydro- α -ergocryptine	4.92	1.12	5.71	1.09	3.84	1.10	8.21	1.13	1.75	1.23
dihydroergocristine	5.50		6.24		4.22		9.26		2.15	1.42
dihydro- β -ergocryptine									3.05	

TABLE 9.9

DEPENDENCE OF CAPACITY RATIOS MEASURED FOR SOME ERGOT ALKALOIDS ON DI-(2-ETHYLHEXYL) PHOSPHORIC ACID (DHP) CONCENTRATION⁶¹

Conditions: μ Bondapak CN column (300x3.9 mm I.D.); eluent flow-rate, 1 cm³/min; detection at 280 nm.

Compound	Eluent composition (%)								
	Hexane	65	65	65	65	65	65	60	70
Chloroform	20	20	20	20	20	20	20	23	17
Acetonitrile	15	15	15	15	15	15	15	17	13
DHP (M)	-	0.0005	0.001	0.005	0.01	0.025	0.005	0.005	
β -Ergocryptinine	0.90	0.89	1.17	4.71	10.4	3.70	4.15	9.67	
α -Ergocryptinine	0.90	0.89	1.17	4.71	10.4	3.70	4.15	9.67	
Ergocorninine	0.90	0.89	1.41	6.46	12.9	4.63	4.48	12.7	
Ergocristinine	1.07	1.07	1.41	7.21	14.3	5.22	5.52	14.6	
β -Ergocryptine	1.07	1.18	1.69	1.96	2.38	2.41	1.48	3.37	
α -Ergocryptine	1.07	1.18	1.69	1.96	2.38	2.41	1.48	3.37	
Ergocornine	1.28	1.36	1.93	2.21	2.77	2.78	1.67	3.89	
Ergocristine	1.41	1.50	2.10	2.21	2.77	2.78	1.67	4.15	
Ergotamine	2.72	2.36	3.62	2.93	3.54	4.11	2.37	5.35	
Ergotaminine	1.48	1.39	5.55	15.9	25.9	16.8	12.0	28.3	
Ergometrine	12.0	12.0	15.2						
Dihydro- β -ergocryptine	2.79	1.75	1.59	1.41	1.69	2.00	1.15	2.70	
Dihydro- α -ergocryptine	2.79	1.75	1.59	1.41	1.69	2.00	1.15	2.70	
Dihydroergocornine	3.28	2.00	1.85	1.71	2.00	2.26	1.30	3.10	
Dihydroergocristine	3.55	2.21	1.97	1.71	2.00	2.26	1.30	3.10	
Dihydroergotamine	4.17	3.71	3.28	2.64	3.00	3.37	1.96	4.63	

TABLE 9.10

INFLUENCE OF DHP CONCENTRATION IN THE PRESENCE OF DEA IN THE ELUENT ON THE CAPACITY RATIOS (k') AND SELECTIVITY FACTORS (r_{ij}) OBTAINED FOR NATIVE AND HYDROGENATED ERGOT PEPTIDE ALKALOIDS⁶¹

Conditions as in Table 9.9

Eluent, hexane-isopropanol (80:20)

Compound	DEA (M) DHP (M)	2×10^{-3} 10^{-3}		10^{-3} 10^{-3}		7.5×10^{-4} 10^{-3}		7.5×10^{-4} 1.5×10^{-3}		7.5×10^{-4} 2×10^{-3}		7.5×10^{-4} 4×10^{-3}	
		k'	r_{ij}	k'	r_{ij}	k'	r_{ij}	k'	r_{ij}	k'	r_{ij}	k'	r_{ij}
Hydrocortisone		1.87		1.85		1.87		1.93		1.87		1.90	
Prednisolone		2.10		2.10		2.01		2.10		2.12		2.13	
β -Ergocryptinine		2.29		2.40		2.63		3.53		5.20		10.4	
α -Ergocryptinine		2.29	1.00	2.40	1.00	2.81	1.07	3.90	1.10	5.70	1.10	11.5	1.11
Ergocorninine		2.94	1.28	3.00	1.25	3.08	1.10	4.50	1.15	6.15	1.08	12.5	1.09
Ergocristinine		3.58	1.22	3.95	1.32	4.41	1.43	6.07	1.35	8.60	1.40	16.9	1.35
β -Ergocryptine		2.06		2.74		3.47		4.53		4.40		4.27	
α -Ergocryptine		2.06	1.00	2.74	1.00	3.81	1.10	5.13	1.13	4.95	1.13	4.87	1.14
Ergocornine		2.42	1.17	3.23	1.18	4.00	1.05	5.13	1.00	4.95	1.00	4.87	1.00
Ergocristine		3.06	1.26	4.48	1.39	5.88	1.47	7.80	1.52	7.51	1.50	7.20	1.48
Ergotamine		4.35		4.90		5.20		7.12		8.35		8.90	
Ergotaminine		4.81	1.11	5.94	1.20	8.40	1.62	12.3	1.72	16.6	1.98	24.5	2.77
Dihydro- β -ergocryptine		2.10		2.87		2.94		3.20		3.26		3.07	
Dihydro- α -ergocryptine		2.10	1.00	2.87	1.00	2.94	1.00	3.20	1.00	3.58	1.10	3.70	1.21
Dihydroergocornine		2.48	1.18	3.20	1.11	3.25	1.10	3.67	1.15	3.87	1.08	4.07	1.10
Dihydroergocristine		3.06	1.23	4.23	1.32	4.31	1.33	5.23	1.43	5.68	1.47	6.03	1.48
Dihydroergotamine		4.06		4.52		4.81		-		6.03		7.13	

TABLE 9.11

CAPACITY FACTORS (k') OF ERGOT ALKALOIDS ON SILICA GEL PACKINGS WITH DIFFERENT ELUENTS^{25,34}Column Lichrosorb Si60, 10 μ m (250x2 mm ID), mobile phase S1 n-hexane - chloroform - ethanol (40:40:10), S2 chloroform - methanol (95:5), S3 chloroform-ethanol (95:5), detection UV 280 or 320 nm

Alkaloid	S1	S2	S3	Alkaloid	S1	S2	S3
Ergometrine maleate	17.41	-	-	Ergocorninine	0.93	0.25	0.13
Ergometrinine	10.00	-	-	Ergocryptinine	0.93	0.25	0.13
Ergotamine tartrate	6.27	-	1.80	Ergocristinine	0.93	0.25	0.13
Ergocornine	1.90	0.60	0.50	Dihydroergocornine ^a	-	1.75	-
Ergocryptine	1.90	0.60	0.50	Dihydroergocryptine ^a	-	1.65	-
Ergocristine	1.90	0.60	0.50	Dihydroergocristine ^a	-	1.50	-
Ergotaminine	1.40	0.37	0.28				

^a As methanesulfonate salt

TABLE 9.12

CAPACITY FACTORS (k') AND SEPARATION FACTORS (α) FOR ERGOT ALKALOIDS ON SILICA GEL PACKINGS WITH DIFFERENT ELUENTS⁵¹Column Lichrosorb Si60, 5 μ m (250x4.6), mobile phase S1 hexane - chloroform - acetonitrile (60:25:15), S2 idem in ratio (56:22:22), S3 idem in ratio (55:20:25), S4 hexane - chloroform - acetonitrile - methanol (55:20:25:3), flow rate 100 ml/h, detection UV 320 nm.

Alkaloid	S1		S2		S3		S4	
	k'	α	k'	α	k'	α	k'	α
β -Ergocryptinine	2.04	1.24	1.00	1.08	0.95	1.05	0.77	
α -Ergocryptinine	2.54		1.08	1.22	1.00		0.77	
Ergocristinine		1.28	1.32	1.06		1.22	0.77	
Ergocorninine	3.24		1.40	1.99	1.22		0.77	
Ergotaminine		2.31	2.78	1.18		2.25	1.01	1.31
β -Ergocryptine	7.48	1.24	3.29	1.12	2.75	1.12	1.21	1.20
α -Ergocryptine	9.28		3.67	1.20	3.09		1.21	
Ergocristine		1.16	4.78			1.15	1.21	
Ergocornine	10.80		4.40		3.54		1.21	
Ergometrinine				4.67			2.11	1.74
Ergotamine			20.54				2.52	1.19
Ergometrine							5.84	2.32

TABLE 9.13

CAPACITY FACTORS OF SOME ERGOT ALKALOIDS ON A POROUS POLYSTYRENE STATIONARY PHASE⁴¹Column Hitachi Gel no 3011-0, 5-7 μ m (500x4.6 mm ID), mobile phase S1 n-hexane - ethanol - triethylamine (70:30:0.5), S2 cyclohexane - ethanol - triethylamine (70:30:0.5), S3 n-hexane - chloroform - triethylamine (5:95:0.5), detection UV 280 nm.

Alkaloids	k' in S1	S2	S3
Ergometrine	8.68	5.15	26
α -Ergocryptine	1.47	0.73	0.76
Ergocornine	1.85	0.88	0.84
Ergocristine	3.07	1.31	1.11
α -Ergocryptinine	1.78	0.89	2.14
Ergocorninine	2.45	1.15	2.86
Ergocristinine	4.31	1.82	3.23
Ergotaminine	5.57	2.41	3.41
Dihydro- α -ergocryptine	1.13	0.57	1.53
Dihydroergocornine	1.48	0.71	1.96
Dihydroergocristine	2.38	1.00	2.37

Jane⁹ separated a wide range of drugs of abuse on microparticulate silica gel with polar mobile phases containing ammonium nitrate solutions. LSD could be analyzed with methanol - 0.2 M ammonium nitrate solution (3:2)(Fig.9.6). The system has been successfully applied to the analysis of LSD^{13,15,16,28,45,46}, but for the analysis of ergot alkaloids the above mentioned reversed-phase system gave better results^{28,46} (Table 9.5).

Szepesy et al.^{25,34,35} found microparticulate silica gel to be very useful for the separation of the alkaloids present in the ergometrine, ergotamine, ergotoxine and dihydroergotoxine groups. The stereoisomers could be separated on silica gel (Table 9.11). To obtain a good separation of the various alkaloids within each group, reversed-phase chromatography was found to be best (Table 9.1). Later⁵¹, a straight-phase system was developed for the separation of the ergotoxine alkaloids (Table 9.12, Figs. 9.7 and 9.8). By means of a solvent system consisting of hexane - chloroform - acetonitrile - methanol (50:20:25:3), a rapid group separation could be achieved (Fig.9.9).

Aigner et al.¹⁴ investigated the separation of some drugs, e.g. ergotamine and ergotamine, on silver iodide impregnated silica gel. Multi-component drugs could be separated with chloroform - diethylamine as mobile phase on a 1.09% silver iodide impregnated silica gel column.

Quercia et al.¹¹ used a microparticulate aluminium oxide column to separate the dihydroderivatives of some ergot alkaloids and pentane - methanol (98:2) or (97:3) as mobile phase.

Yoshida et al.⁴¹ isolated ergotoxine and ergotoxinine from ergot by means of a silica gel column and cyclohexane - acetone (1:1) as mobile phase. The ergotoxinine group of alkaloids was separated on a porous polystyrene, modified by hydroxymethyl groups by using *n*-hexane - ethanol - triethylamine (70:30:0.5) as mobile phase. The ergotoxine group alkaloids and their dihydro derivatives could be separated in this system (Table 9.13).

For the LC-MS analysis of clavine alkaloids in ergot fermentation broth, Eckers et al.^{57,58} separated the alkaloids on silica gel with the mobile phase dichloromethane - methanol - concentrated ammonia (95:5:0.1)(Fig.9.10).

9.4. DETECTION

The intense fluorescence of LSD provides the basis for a very sensitive and selective detection of this compound - 10 pg. The excitation wavelength used is about 325 nm^{13,15,16,21,28,53} and the emission is measured at 389 nm⁵³, 400 nm^{4,28} or 420 nm^{13,15,16,21}. Prolonged irradiation - 5 min - of LSD trapped in a scanning fluorimetric detector, results in the conversion into a non-fluorescent lumi-derivative. The disappearance of the fluorescence upon UV-irradiation has been used to distinguish LSD from other fluorescent compounds that do not exhibit this behaviour^{12,27,28}. The same principle has been used for the identification of ergot alkaloids by Scholten and Frei⁴⁴. A photochemical reaction detector was designed in which the eluted ergot alkaloids were irradiated for 20 sec with 327 nm UV-light - and the emission was measured at 410 nm. Dihydroderivatives were irradiated at 280 nm and the fluorescence measured at 340 nm. Under such conditions a 90-99% decrease of the fluorescence was found for the 17 alkaloids investigated.

The influence of the solvent on the quenching of the fluorescence was studied by Heacock et al.². From Table 9.14 it is clear that only chloroform causes considerable quenching. The fluorescence maxima of some ergot alkaloids are listed in Table 9.15².

TABLE 9.14

INFLUENCE OF SOLVENT ON FLUORESCENCE INTENSITY OF ERGOTAMINE ($4.5 \times 10^{-3} \text{M}$)²
 $\lambda_{\text{ex}} = 350 \text{ nm}$

Solvent	Relative intensity(%)
100% Ethanol	100
50% Diisopropyl ether ^a	130
50% Cyclohexane ^a	113
50% Acetone ^a	96
50% Hexane ^a	91
50% Benzene ^a	109
50% Chloroform ^a	13

^a 50% mixtures with absolute ethanol

TABLE 9.15

EMISSION MAXIMA AND RELATIVE FLUORESCENCE INTENSITIES FOR SOME ERGOT ALKALOIDS IN ACETONE SOLUTION².

$\lambda_{\text{ex}} = 350 \text{ nm}$, concentration = 100 ppm.

Alkaloid	λ_{max} emission	Relative intensity(%)
Ergotamine	400	40.2
Ergotaminine	397	100.0
Ergocristine	400	21.0
Ergocryptine	403	22.1
Ergocryptinine	397	62.2
Ergocornine	402	18.3
Ergocorninine	398	83.8
Ergosine	398	56.1
Lysergic acid amide ^b	393	1.1
Isolysergic acid amide	393	61.5
D-Lysergic acid	392	32.8
Ergotrate	403	28.9
Isosetoclavine ^b	393	82.2
Elymoclavine ^b	394	0.3
Agroclavine	392	0.5
D-Lysergic acid diethylamide	397	62.2

^b Concentration = 20 ppm, due to low solubility of compound in acetone.

Fluorescence detection has found wide application in the analysis of ergot alkaloids^{7,23, 36,50}. Scott and Lawrence⁵⁰ found that for the fluorimetric detector used in their investigations, the optimum wavelength for excitation was 235 nm. Baker et al.⁴ compared a fluorimetric detector and detectors with variable wavelength (334 nm) and single wavelength (254 nm) for the analysis of illicit LSD samples. The fluorimetric and the variable wavelength detection allowed analysis without sample pretreatment, whereas several peaks interfered with LSD by detection at 254 nm.

For a specific detection of the ergotoxine alkaloids, Szepesy et al.²⁵ used a wavelength of 320 nm, for their dihydroderivatives 280 nm. Bethke et al.¹⁷ applied simultaneous detection at 280 and 320 nm for ergotamine and its decomposition products. Detection at 241 nm has also been employed⁵².

Since the lumi alkaloids have only very small absorption at 320 nm, they do not interfere with the detection of the ergot alkaloids with a 9,10-double bond. The dihydro and the lumi

alkaloids can be detected at 280 nm, the lumi alkaloids with an increase of sensitivity by a factor of 1000 when compared with the 320 nm detection limit. Szepeszy et al.²⁵ used the wavelength 320 nm and 280 nm for the detection of the ergotoxine and the dihydroergotoxine alkaloids, respectively.

For quantitative determinations of ergot alkaloids, Wurst et al.^{29,43} made use of several wavelengths - 310, 282, 254, 240 and 225 nm. In that way, partially resolved alkaloids could be quantified.

White⁴⁵ developed an electrochemical detection method for the HPLC, also suitable for the analysis of LSD and some phenothiazine derivatives. Santi et al.^{10,26} as well as Gfeller et al.^{30,38} studied the selective detection of ergotamine as picrate ion-pair in multi-component preparations. This method is discussed in Chapter 4. Frei⁴⁰ has reviewed reaction liquid chromatography, including the method mentioned above.

A post column fluorescent ion-pairing technique has been developed for ergotamine⁴². The major advantage of this technique is an improvement of the selectivity, as no interfering peaks due to non-ion-pairing compounds are observed in urine analyses (see Chapter 4).

Lawrence et al.⁴⁸ described a similar technique, but they used an organic eluent in combination with a silica gel column, instead of an aqueous eluent as used by Gfeller et al.⁴² (see Chapter 4).

Eckers et al.^{57,58} reported the analysis of clavine alkaloids in ergot fermentation broth by means of LC-MS (Fig.9.10). In general, EI-MS was found to give more informative results than CI-MS; however, the alkaloids palliclavine decomposed during EI-MS, necessitating the latter method for the analysis of that alkaloid. Various conditions for the mass spectrometry were studied.

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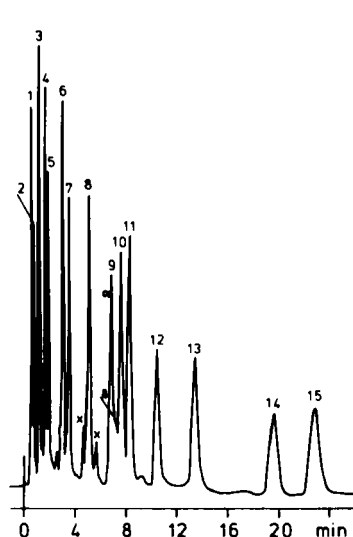


Fig. 9.1. Separation of some ergot alkaloids¹⁹
Column Lichrosorb RP8 5 μ m (125x4.2 mm ID), mobile phase acetonitrile - 0.02% aqueous ammonium carbonate (2:3), flow rate 100 ml/h, detection UV 254 nm. Peaks: 1, lysergic acid; 2, isolysergic acid; 3, lysergic acid amide and ergometrine; 4, isolysergic acid amide; 5, ergometrine; 6, ergosine; 7, ergotamine; 8, ergocornine; 9, ergocryptine (α and β); 10, ergocristine; 11, ergosinine; 12, ergotamine; 13, ergocornine; 14, ergocryptine; 15, ergocristine; x, unknown. (reproduced with permission from ref. 19, by the courtesy of Friedr. Vieweg & Sohn, Wiesbaden)

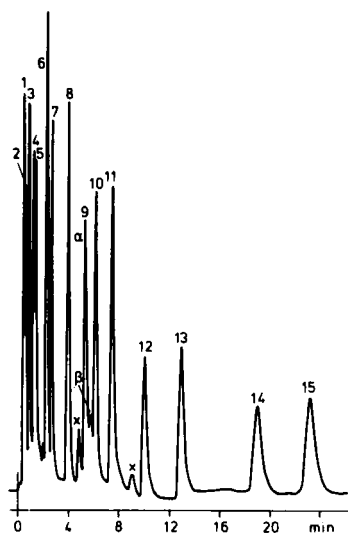


Fig. 9.2. Separation of some ergot alkaloids¹⁹
Column Lichrosorb RP18 10 μ m (125x4.2 mm ID), mobile phase acetonitrile - 0.02% aqueous ammonium carbonate (42:58), flow rate 100 ml/h, detection UV 254 nm. Peak numbering as in Fig. 9.1. (reproduced with permission from ref. 19, by the courtesy of Friedr. Vieweg & Sohn, Wiesbaden)

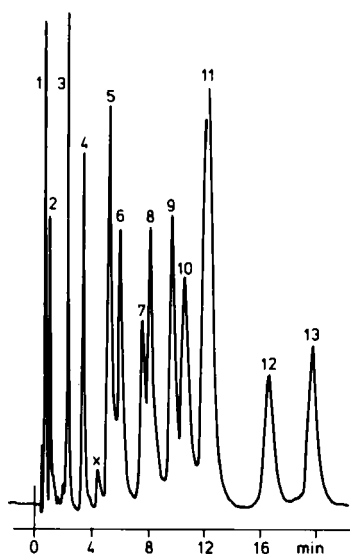


Fig. 9.3. Separation of some ergot alkaloids¹⁹
Column Spherisorb ODS 5 μ m (125x4.2 mm ID), mobile phase acetonitrile - 0.02% aqueous ammonium carbonate (42:58), flow rate 100 ml/h, detection UV 254 nm. Peak numbering 1-8 as in Fig. 9.1., peak 9, ergosinine; 10, ergocryptine; 11, ergocristine, ergocornine and ergotamine; 12, ergocryptine; 13, ergocristine; x, unknown. (reproduced with permission from ref. 19, by the courtesy of Friedr. Vieweg & Sohn, Wiesbaden)

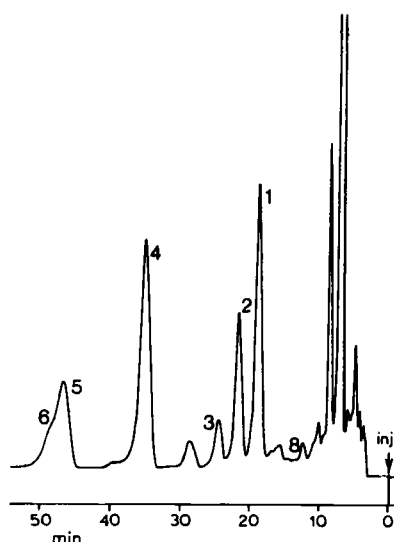
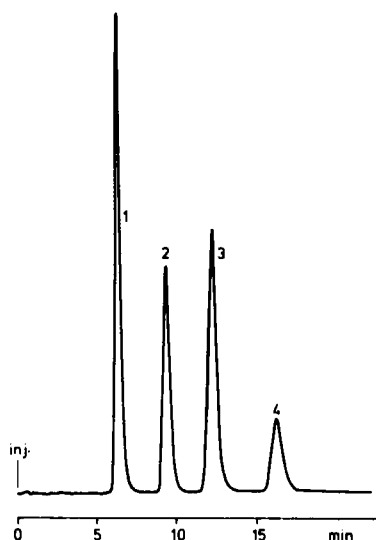


Fig. 9.4. Separation of dihydroergotoxine alkaloids³³

Column Lichrosorb RP18 5 μ m (150x3 mm ID), mobile phase water - acetonitrile - triethylamine (32:8:1), flow rate 1.0 ml/min, detection UV 280 nm. Peaks: 1, dihydroergocornine; 2, dihydro- α -ergocryptine; 3, dihydroergocristine; 4, dihydro- β -ergocryptine. (reproduced with permission from ref. 33, by the courtesy of Journal Pharmaceutical Sciences)

Fig. 9.5. Analysis of ergocornine and ergocryptine in fermentation liquor⁶⁰

Column Lichrosorb RP18 10 μ m (250x4.6 mm ID), mobile phase tetrahydrofuran - 0.01 M aqueous ammonium acetate (2:3), flow rate 1.5 ml/min, detection UV 322 nm. Peaks: 1, ergocornine; 2, α -ergocryptine; 3, β -ergocryptine; 4, ergocorninine; 5, α -ergocryptinine; 6, β -ergocryptinine; 7, ergometrine; 8, ergometrinine.

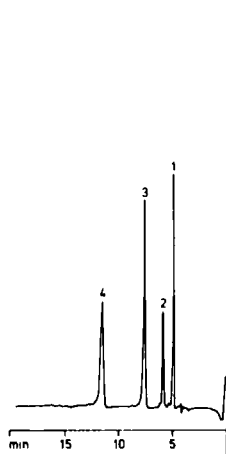


Fig. 9.6. HPLC analysis of LSD and some related compounds⁹

Column Partisil 6 μ m (250x4.6 mm ID), mobile phase methanol - 0.2 M aqueous ammonium nitrate (3:2), flow rate 1 ml/min, detection UV 320 nm. Peaks: 1, lysergic acid; 2, lysergamide; 3, LSD; 4, isoLSD.

Fig. 9.7. Separation of ergotoxine alkaloids⁵¹

Column Lichrosorb Si60 5 μ m (250x4.6 mm ID), mobile phase hexane - chloroform - acetonitrile (56:22:22), flow rate 100 ml/min, detection UV 320 nm. Peaks: 1, β -ergocryptinine; 2, α -ergocryptinine; 3, ergocorninine; 4, β -ergocryptine; 5, α -ergocryptine; 6, ergocornine; x, unknown.

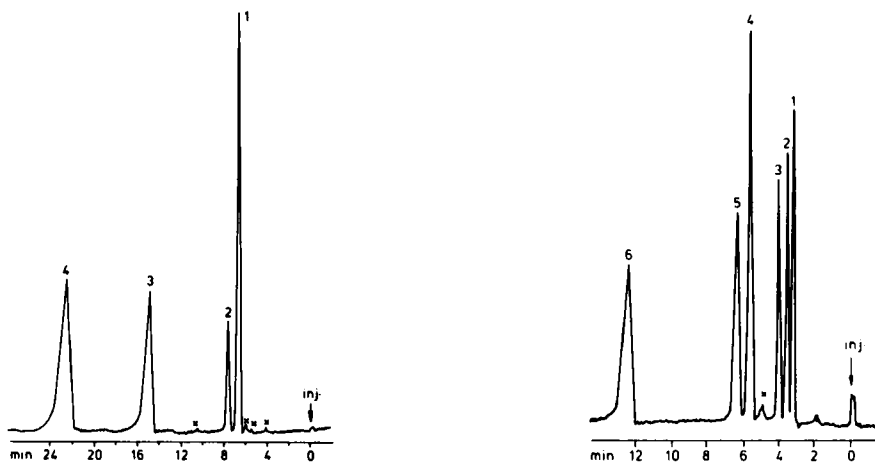


Fig. 9.8. Separation of four isomers of ergocristine⁵¹

Column Lichrosorb Si60 5 μ m (250x4.6 mm ID), mobile phase hexane - chloroform - acetonitrile (56:22:22), flow rate 100 ml/h, detection UV 320 nm. Peaks: 1, ergocristinine; 2, aci-ergocristinine; 3, ergocristine; 4, aci-ergocristine; x, unknown.

Fig. 9.9. Separation of ergot alkaloids⁵¹

Column Lichrosorb Si60 5 μ m (250x4.6 mm ID), mobile phase hexane - chloroform - acetonitrile methanol (55:20:25:3), flow rate 100 ml/h, detection UV 320 nm. Peaks: 1, ergocristinine, ergocorninine, α - and β -ergocryptinine; 2, ergotaminine; 3, ergocristine, ergocorninine, α - and β -ergocryptine; 4, ergometrinine; 5, ergotamine; 6, ergometrine; x, unknown.

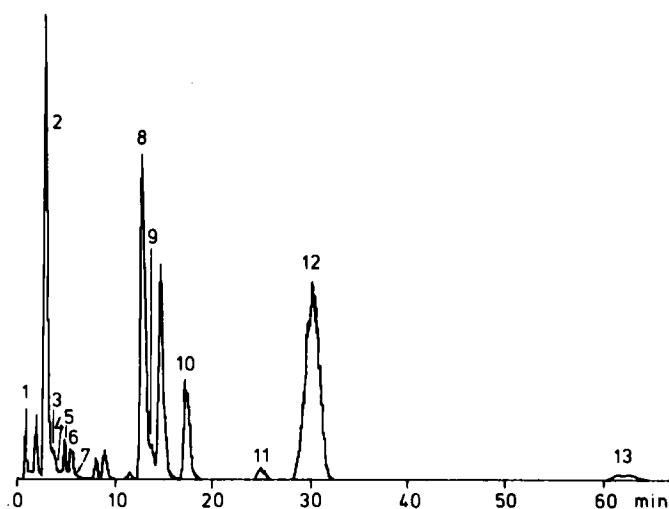


Fig. 9.10. Reconstructed total ion current trace obtained by EI LC/MS of ergot extract^{57,58}

Column Spherisorb 5W silica gel (250x5 mm ID), mobile phase dichloromethane - methanol - concentrated ammonia (95:5:0.1), flow rate 1 ml/min. Peaks: 1, solvent front; 2, agroclavine; 3, setoclavine; 4, festuclavine; 5, palliclavine or isomer; 6, palliclavine or isomer; 7, N-noragroclavine; 8, elymoclavine; 9, penniclavine; 10, isochanoclavine; 11, norchanoclavines I and II; 12, chanoclavine I; 13, chanoclavine II. (reproduced with permission of John Wiley & Sons, Ltd.)

TABLE 9.16

HPLC ANALYSIS ERGOT ALKALOIDS IN AND FROM FUNGI

ALKALOIDS *	AIMS	STATIONARY PHASE	COLUMN DIM. LxID mm	MOBILE PHASE	REF.
Lysac, Ergta,Ergtaine,Ergct,Ergctine diHErgct,Ergcp,Ergcpine, diHErgcp,Ergco,Ergcoine, diHErgco,Ergm,Ergmine	Analysis plant extracts,fermented products and pharmaceutical preparations (Table 9.1 and 9.11)	Lichrosorb Si60,10 μ m Lichrosorb RP2,RP8 and RP18	250x2 250x2	n-hexane-CHCl ₃ -EtOH(4:4:1) -CHCl ₃ -MeOH(95:5) CHCl ₃ -EtOH(95:5) ACN-0.01M (NH ₄) ₂ CO ₃ (2:3)	25,34
Agcl,Chcl,Elcl,Fcl,Pcl,Pycl, Scl,isoScl,paspaclavine, paliclavine,Lysam,isoLysam, Lysgol,Lysg,lysergen	Analysis clavines and simple lysergic acid derivatives (Table 9.6)	Micropak NH ₂ ,10 μ m	250x2	CHCl ₃ -isoprOH(9:1),(8:2) Et ₂ O-isoprOH(7:3),(6:4) Et ₂ O-EtOH(84:16),(8:2) also gradient elution	29
Ergta,Ergtaine,Ergct,Ergctine	Analysis in crude Ergot	Micropak Si-10	250x2	CHCl ₃ -EtOH(95:5)	35
Ergta,Ergtaine,Ergs,Ergsine, Ergct,Ergctine,Ergcp,Ergcpine, Ergco,Ergcoine,Ergm,Ergmine papaverine	Analysis in sclerotia,culture media and pharmaceutical pro- ducts (Table 9.3)	Lichrosorb RP18,10 μ m	250x4	0.1% (NH ₄)OAc in ACN-H ₂ O (35:65)	36
Ergtaine,Ergct,Ergctine, diHErgct, α -Ergcp, α -Ergcpine, diHErgcp,Ergco,Ergcoine, diHErgco,Ergm	Analysis in crude Ergot and phar- maceutical preparations (Table 9.13)	Hitachi Gel no 3301-0 5-7 μ m	500x4.6	n-hexane-EtOH-TrEA(70:30:0.5) n-hexane-CHCl ₃ -TrEA(5:95:0.5) cyclohexane-EtOH-TrEA(70:30:0.5)	41
Agcl,Lysam,isolysam Ergta,Ergtaine,8-OH-Ergta, Ergs,Ergsine,Ergst,Ergstine, Ergct,Ergctine,Ergcp,Ergcpine, Ergco,Ergcoine,Ergm,Ergmine	Analysis in fermentation media and pharmaceutical preparations (Table 9.7)	Lichrosorb NH ₂ ,10 μ m or Micropak NH ₂ , 10 μ m	250x2	CHCl ₃ -isoprOH(9:1) Et ₂ O-isoprOH(6:4) Et ₂ O-EtOH(84:16),(88:12),(93:7) also gradient elution	43
Ergta,Ergtaine,Ergs,Ergsine, Ergct,Ergctine,Ergcp,Ergcpine, Ergco,Ergcoine,Ergm,Ergmine	Analysis ergot alkaloids in flour	Lichrosorb RP8, 5 μ m	250x4.6	ACN-0.02M aq.(NH ₄) ₂ CO ₃ (43:57), (35:65),(28:72)	50
Ergta,Ergtaine,Ergct,Ergct- ine, α -, β -Ergcp, α -, β -Ergcpine, Ergco,Ergcoine,Ergm,Ergmine	Analysis ergotoxine alkaloids (Table 9.12 and Fig.9.7,9.8 and 9.9)	Lichrosorb Si60,5 μ m	250x4.6	Hexane-CHCl ₃ -ACN(56:22:22), (60:25:15),(55:20:25) Hexane-CHCl ₃ -ACN-MeOH (55:20:25:3)	51
Ergta,Ergtaine,Ergct,Ergcti- ne, α -Ergcp, β -Ergcp,Ercpine, Ergco,Ergcoine	Analysis in sclerotium material	Silica gel C8, 5 μ m	250x4	ACN-H ₂ O(55:45) containing 0.04%(NH ₄)CO ₃	52

*For abbreviations see footnote Table 9.19

Agcl,norAgcl,Chcl I and II, isoChcl I,norChvl I and II, Elcl,Fcl,Pcl,Pycl,ScI,isoScI, norisoScI,palliclavine	Analysis in ergot fermentation- broth with LC-MS (Fig.9.10)	Spherisorb 5W ODS-type 5 μ m NH ₂ -type, 5 μ m	250x5 250x5 not given	CH ₂ Cl ₂ -MeOH-conc.NH ₄ OH (95:5:0.1) MeOH-H ₂ O-conc.NH ₄ OH (60:40:0.1) isooctane-CH ₂ Cl ₂ -MeOH(5:4:1)	57,58
Ergm,Ergmine,Ergco,Ergcoine, α -Ergcp, β -Ergcpine, β -Ergcp, α -Ergcpine,diHErgco,diH- α - Ergco,diH- β -Ergcp	Separation (Fig.9.5,Table 9.4)	Lichrosorb RP18, 10 μ m	250x4.6	THF-0.01M aq NH ₄ OAc(2:3)	60
16 Ergot alkaloids	Separation with straight phase ion-pair HPLC (Tables 9.9, 9.10)	μ Bondapak CN	300x3.9	Hexane-CHCl ₃ -ACN-di-(2-ethyl- hexyl)phosphoric acid in various ratios	61

TABLE 9.17

HPLC ANALYSIS ERGOT ALKALOIDS IN PHARMACEUTICAL PREPARATIONS

ALKALOIDS*	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
Agcl,Elcl Pcl,isoScI,LSD, Lysg,isoLysg,Ergta, Ergtaine,Ergs, Ergsine,Ergct, Ergctine,Ergcp, Ergcpine,Ergco, Ergcoine,Ergm		Preliminary investigation of separation with HPLC	Corasil	100x2.4	CHCl ₃ -MeOH-EtOAc-AcOH(60:20:50:3) CHCl ₃ -MeOH(100:4)	2
diHErgct,diHErgcp, diHErgco		Separation on small particle size column packings	μ Bondapak C18	300x4	ACN-0.01M aq.(NH ₄) ₂ CO ₃ (2:3)	6
Ergta,atropine, scopolamine		Separation as ion-pairs	Spherosil X0B,5-10 μ m loaded with 0.03M pi- cric acid and buffer pH 5 Silica gel 100, 5 μ m loaded with 0.06M pi- cric acid and buffer pH 5	100x2.8 100x2.8	CHCl ₃ sat.with 0.05M picric acid in pH 5 buffer CHCl ₃ sat.with 0.06M picric acid in pH 5 buffer	10
diHErgta,diHErgct, diHErgcp,diHErgco		Analysis in pharmaceutical preparations	Micropak A1-5	250x2	Pentane-MeOH(98:2),(97:3)	11

*For abbreviations see footnote Table 9.19

Ergta,Ergtaine atropine,scopo- lamine,caffeine	Butalbital, phenobarbital	Separation on silver im- pregnated silica gel	Lichrosorb Si100,5 μ m imp.with 1.09% AgI	not given	CHCl ₃ -DEA(99.99:0.01) gradient A CHCl ₃ -hexane(1:1) B CHCl ₃ -MeOH-DEA(90:10:0.5) linear gradient from 16 to 92% B in A (1.5-2.5 min)	14
Ergta,Ergtaine, aciErgta,aciErg- taine,Lysam,iso- Lysam,Lysac,iso- Lysac,lumiErgta		Quality control ergotamine preparations (Table 9.2)	μ Bondapak C18 or Nucleosil C18,10 μ m	300x4 300x4.6	ACN-0.01M(NH ₄) ₂ CO ₃ (2:1),(1:1)	17
Ergta,Ergtaine aciErgta,aciErgtaine Lysam,isoLysam, Lysac,isoLysac		Separation with stepwise gradient system	Nucleosil C18 5 μ m	150x3	ACN-0.01M(NH ₄) ₂ CO ₃ stepwise gradient: 8%,15%,30%, 40%,50% and 60%	18
Ergta,Ergtaine,Ergs, Ergsine,Ergct,Erg- ctine,diHErgct, α -, β -Ergcp,Ergcp- ine,diHErgcp, Ergco,Ergcoine, Ergm,Ergmine, Lysam,isoLysam, Lysac,isoLysac		Separation with reversed phase HPLC (Fig.9.1,9.2, 9.3)	Spherisorb ODS,5 μ m	125x4.2	ACN-0.02%aq(NH ₄) ₂ CO ₃ (42:58)	19
Ergct, α -, β -Ergcp, Ergco		Influence of organic bases on the stability reversed phase stationary phases	Lichrosorb RP18,5 μ m	250x4.6	0.05M TrEA in ACN-H ₂ O(1:1)	24
Ergta,Ergtaine, Ergct,Ergctine, diHErgct, Ergcp,Ergcpine, diHErgcp Ergco,Ergcoine, diHErgco, Ergm,Ergmine,Lysac		Analysis pharmaceutical preparations (Table 9.1 and 9.10)	Lichrosorb Si60,10 μ m	250x2	n-hexane-CHCl ₃ -EtOH(4:4:1) CHCl ₃ -MeOH(95:5) CHCl ₃ -EtOH(95:5)	25,34
Ergta,Ergtaine, diHErgta,tropane alkaloids,caffeine	butalbital,pheno- barbital,barbit- al,pizotifene	Separation with ion-pair chromatography	Lichrosorb Si100,5 μ m loaded with 0.06M pi- cric acid and buffer pH 6	150x3	CHCl ₃ sat.with 0.06M picric acid and buffer pH 6	26,30, 38
Ergm,Ergmine, Lysac		Analysis ergometrine preparations	μ Bondapak C18	300x4	ACN-AcOH-H ₂ O(20:1:79)	31

diHErgct, diH α -Ergcp, diH β -Ergcp, diHErgco		Separation dihydroergo- toxine in pharmaceutical preparations (Fig.9.4)	Lichrosorb RP18, 5 μ m	150x3	H ₂ O-ACN-TrEA(32:8:1) H ₂ O-MeOH-TrEA(25:3.6:1)	33
diHErgta, diHErgct, diH α -Ergcp, diH β -Ergcp, diHErgco		Analysis in pharmaceutical preparations (Table 9.8)	Lichrosorb RP18, 10 μ m Lichrosorb RP8, 7 μ m	250x4 250x4.6	ACN-H ₂ O-DEA(375:625:21) ACN-H ₂ O(45:60)+0.04g(NH ₄) ₂ CO ₃ ACN-H ₂ O-TrEA(45:60:0.4)+citric acid(0.166g), pH 7.1 ACN-H ₂ O-TrEA(45:60:1)+0.3g sodium acetate, pH 7.1 ACN-H ₂ O(45:60)+0.1g tetradecyl trimethylammonium bromide pH 7.1	39
Ergtaine, Ergct, Ergctine, diErgct, α -Ergcp, α -Erg- cpine, diH α -Ergcp Ergco, Ergcoine, diHErgco, Ergm		Analysis in pharmaceutical preparations (Table 9.13)	Hitachi Gel no 3011-0 5-7 μ m	500x4.6	n-hexane-EtOH-TrEA(70:30:0.5) n-hexane-CHCl ₃ -TrEA(5:95:0.5) Cyclohexane-EtOH-TrEA(70:30:0.5)	41
diHErgta, bromo- cryptine, atropine, emetine, ephedrine	Pindolol, guanfa- cin, ketotifen, pizotifen, cle- mastine	Post column derivatization using the fluorimetric ion-pair technique	Lichrosorb DIOL, 10 μ m Lichrosorb RP8, 10 μ m	250x4 100x4.6	0.1M phosphate buffer (pH 3) MeOH-0.02M aq. phosphate buffer (pH 3)(3:2)	42
Ergta, Ergtaine, aciErgta, aciErg- taine, Ergct, Ergcp, Ergco		Detection with a photo- chemical reaction detector	Lichrosorb RP18, 5 μ m	120x4.6	ACN-0.01M NaHCO ₃ (42:58), (38:62), pH 2.2 or 8.5	44
diHErgta		Stability studies	μ Bondapak C18	300x4	ACN-H ₂ O(2:3)	47
Ergta, atropine	Hydroxyatrazine	Detection ion with post- column derivatization	Lichrosorb Si60, 5 μ m	60x3	0.1M butyric acid in CHCl ₃ - MeOH(9:1)	48

TABLE 9.18

HPLC ANALYSIS LSD AND RELATED COMPOUNDS IN DRUG SEIZURES AND AS PURE COMPOUNDS

ALKALOIDS*	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM LxID(mm)	MOBILE PHASE	REF.
LSD, isoLSD, Lysac, Lysam, isolysam, Ergta, Ergtaine, diHErgta, Ergct, Ergctine, diHErgct, Ergcp, Ergm, MeErgm, MeMeErgm	STP, strychnine, phencyclidine	Analysis illicit preparations	Sil-X Corasil II	500x2.3 610x2.3	ACN-(isopr) ₂₀ (4:6) ACN-(isopr) ₂₀ (25:75)	1
LSD, isoLSD, Lysac, Lysam, Lysgol, Ergta, Ergs, Ergsine, Ergct, Ergctine, diHErgct, Ergcp, Ergcpine, diHErgcp, Ergco, diHErgco, Ergm, Ergmine, MeErgm, MeMeErgm		Identification LSD in illicit preparations by HPLC and GLC	Corasil C18	1200x2.2	MeOH-0.1% aq. (NH ₄) ₂ CO ₃ (3:2)	3,8
LSD		Comparison of photometric detectors for HPLC	Zorbax Sil	250x2.1	CH ₂ Cl ₂ -MeOH-AcOH(70:30:0.1)	4
LSD, codeine, heroin, methadon, cocaine, strychnine, mescal- ine, quinine	Barbiturates, am- phetamines, various other drugs of abuse	Identification street drugs	Corasil II, 37-50 µm	500x2.3	Cyclohexane-cyclohexyl- amine (98.8:0.2) gradient elution (linear) A to B A skelly B-95% EtOH-dioxane- cyclohexylamine (99.1:50:25:13) B idem (686:100:200:14)	5
LSD, isoLSD, Lysac, Lysam		Separation drugs of abuse (Fig.9.6)	Partisil 6 µm	250x4.6	MeOH-0.2 M NH ₄ NO ₃ (3:2)	9
LSD		Analysis illicit samples	Partisil 5 µm	250x4.9	MeOH-0.3% aq. (NH ₄) ₂ CO ₃ (3:2)	16
LSD, isoLSD, Lysac, Lysam, Ergta, Ergm	Phencyclidine, N-methylpropyl- amide, various other drugs of abuse	Ion-pair chromatography for the separation of the drugs of abuse	µBondapak C18	300x4	0.005M heptanesulfonic acid in MeOH-AcOH-H ₂ O (40:1:59), pH 3.5	20

*For abbreviations see footnote Table 9.19

LSD		Fluorescence detection for HPLC-analysis LSD	Micropak MCH-10	250x2.2	ACN-0.1M aq. (NH ₄) ₂ CO ₃ (1:1)	21
LSD		Photochemical detection for identification LSD	Spherisorb ODS	100x4.6	MeOH-aq. 0.025M Na ₂ HPO ₄ (65:35) pH 8	27,12
LSD, isoLSD		Semipreparative HPLC for the identification of drugs of forensic interest	μBondapak C18	300x4.4	0.005M methanesulfonic acid in MeOH-AcOH-H ₂ O(40:1:59), pH 3.5	32, 49
			Partisil-10 ODS	250x9.4	0.04M methanesulfonic acid in MeOH-AcOH-H ₂ O(40:1:59), pH 3.5	32, 49
LSD, lysergic acid methyl-propylamide	Benzocaine	Analysis illicit preparations	μBondapak C18	300x4	ACN-H ₂ O-1% aq. (NH ₄) ₂ CO ₃ (400:572:28)	37
LSD	Phenothiazine type drugs	Electrochemical detector	Silica C Syloid 74, 7 μm	200x4.6	MeOH- pH 10.2 NH ₄ NO ₃ buffer (9:1)	45
LSD, isoLSD, Lysac, Lysam, Lysgol, Ergta, diHErgta, Ergs, Ergsine, Ergct, diHErgct, Ergcpine, diHErgcp, Ergco, diHErgco, Ergm, MeErgm, MeMeErgm		Analysis illicit preparations with TLC, HPLC and MS (Table 9.5)	Sherisorb 5 ODS	100x4.6	MeOH-0.025M Na ₂ HPO ₄ (65:35) pH 8	
			Spherisorb S5W	150x4.6	MeOH-0.2M NH ₄ NO ₃	

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TABLE 9.19

HPLC ANALYSIS ERGOT ALKALOIDS IN BIOLOGICAL MATERIAL

ALKALOIDS *	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM LxID(mm)	MOBILE PHASE	REF.
Ergtaine	Reserpine	Determination in plasma	Silica gel 10 μm	250x2.2	(isopr) ₂ O-ACN-MeOH (69.5:30:0.5)	7
LSD, isoLSD		Detection in biological fluids, in combination with TLC	Partisil 6 μm	250x4.6	MeOH-0.2M aq. NH ₄ NO ₃ (11:9)	15,13
diHErgct, diHErgcp, diHErgco		Trace enrichment technique	Nucleosil C18, 5 μm	100x3	ACN-0.1M aq. (NH ₄) ₂ CO ₃ (2:3)	22
Ergta	Quinine	Determination in intestinal homogenate	Partisil 10/25 ODS	250x4.6	ACN-1% acetate buffer pH 6.5 (55:45)	23

D-LSD, isoLSD, Lysac, Lysam, Lysgol, lumiLSD, 2-oxoLSD, Lysac monoethylamide, Ergta, diHErgta, Ergs, Ergsine, Ergct, diHErgct, Ergcp, Ergcpine, diHErgcp, Ergco, diHErgco, Ergm, Ergmine, MeErgm, MeMeErgm, Ergothioneine		Analysis LSD in body fluids (Table 9.5)	Spherisorb 5 ODS	100x4.6	MeOH-0.025M Na ₂ HPO ₄ (65:35) pH 8	
			Spherisorb S5W	150x4.6	MeOH-0.2M NH ₄ NO ₃ (3:2)	
						28
diHErgta, bromo-cryptine, metine, hyoscyamine, ephedrine	Various drugs	Detection with post-column fluorimetric ion-pair technique	Lichrosorb RP8	100x4.6	MeOH-0.02M phosphate buffer (pH 3) (3:2)	
			Lichrosorb Diol	250x4.6	0.1M phosphate buffer (pH 3)	42
Ergta, Ergtaine, MeErgm, MeMeErgm, Ergct		Analysis in plasma	Hypersil ODS, 5 µm	250x4.6	ACN-0.01M (NH ₄) ₂ CO ₃ (3:7), (1:1)	53
LSD		Detection in serum and urine	Lichrosorb RP8, 7 µm with column switch to Lichrosorb Si 60, 5 µm	250x4 125x4	A MeOH-0.3% KH ₂ PO ₄ (pH=3) (1:1) B MeOH-1% (NH ₄) ₂ CO ₃ (3:2) solvent A for column switch system	62

*Abbreviations used in Tables 9.16 - 9.19:

Agcl	Agroclavine	Lysac	Lysergic acid	Ergstine	Ergostinine
Ccl	Costaclavine	LSD	β-Lysergide	Ergs	Ergosine
Chcl	Chanoclavine	Lysg	Lysergine	Ergsine	Ergosinine
Elcl	Elymoclavine	Lysgol	Lysergol	Ergtox	Ergotoxine (Ergct+Ergcp+Ergco)
Fcl	Festoclavine	Ergm	Ergometrine (ergobasine or ergonovine)	Ergct	Ergocristine
Fucl	Fumiclavine	Ergmine	Ergometrinine	Ergctine	Ergocristinine
Mcl	Molliclavine	Ergta	Ergotamine	Ergcp	Ergocryptine
Pcl	Penniclavine	Ergtaine	Ergotaminine	Ergcpine	Ergocryptinine
Pycl	Pyrroclavine	MeErgm	Methylergometrine	Ergco	Ergocornine
Sccl	Setoclavine	MeMeErgm	1-Methylmethylergometrine (methysergide)	Ergcoine	Ergocorninine
Lysam	Lysergic acid amide (ergine)	Ergst	Ergostine	diH-	dihydro-

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Chapter 10

STEROIDAL ALKALOIDS

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The steroid alkaloids comprise a large number of heterocyclic plant bases - some occurring as the aglycone portion of a glycoalkaloid bonded to one or more hexoses (*Solanum* alkaloids), others as esters (*Veratrum* alkaloids). So far, HPLC has had only limited application in the field of steroid alkaloid analysis - mainly because of the lack of a strong chromophore in most of such alkaloids, thus necessitating RI detection or UV detection at 200-210 nm. A short wavelength, UV detection limits the number elution solvents to those having low UV absorption: water, acetonitrile, ethers, alcohols and hydrocarbons. Fluorescent ion-pairing techniques or derivatization techniques have so far not been used to improve the detection properties of steroid alkaloids.

10.1. HPLC SYSTEMS

Hunter et al.¹ developed a straight-phase preparative HPLC method for some steroid alkaloids isolated from a *Solanum* species. It was later improved by using microparticulate silica gel as stationary phase^{4,6}. The improved method was applied to the separation of a series of non-glycosidic *Solanum* and non-esterified *Veratrum* alkaloids (Figs.10.1 and 10.2). Prior to the HPLC analysis the *Solanum* alkaloids were fractionated by means of a chromatofuge⁶.

Reversed-phase HPLC of potato steroid glycoalkaloids was reported by Bushway et al.^{2,5}. An octadecyl stationary phase was used to separate the glycoalkaloids according to the number of glycosidic bonded hexoses in the molecule, and a stationary phase containing alkylamino groups was used to separate the glycoalkaloids α -chaconine, β -chaconine and α -solanine (Fig. 10.3). However, for the analysis of the glycoalkaloids in potato tubers, peels, and sprouts, a "carbohydrate analysis column" was preferred, since no interfering peaks were observed (Fig. 10.4). The same kind of column, as well as an octadecyl column, were used by Crabbe and Fryer³ for the separation of solasodine, solasodine glycoalkaloids and solasodine. The influence of varying ratios of the solvents on the k' was investigated. Buffered solvent systems (pH 7-7.5 with 0.01 M Tris buffer) were found to give better reproducibility. Glycoalkaloids of the solasodine and solasodine types were separated on the octadecyl column. Increasing the water content in the mobile phase from 10% to 25% led to separation of the glycoalkaloids in three groups: mono-, di- and tri-glycosidic alkaloids (Fig.10.5). On replacing methanol by acetonitrile in the mobile phase, an increase of the selectivity and of the number of theoretical plates was achieved. The composition of the solvent had, in such a case, a greater effect on the k' than in the case of methanol - water systems. The "carbohydrate analysis column" behaved as a normal-phase column and gave increased retention with increased polarity of the alkaloids. This column could be used to separate alkaloids with the same number of glycosidic bonded hexoses (Fig.10.6).

The amino-type stationary phase was also used in more recent studies by Bushway^{8,9,10}. For the separation of α -chaconine and α -solanine in potato extracts, the mobile phase tetrahydrofuran - acetonitrile - water - methanol (50:25:15.5:9.5) was used, resulting in a considerably reduced analysis time when compared to the previously reported methods. For analysis of the alkaloids and their metabolites, the mobile phase ratio was changed to (55:30:10:5)(Fig.10.7). For separation of the lower glycosides from α -chaconine and α -solanine, a carbohydrate analysis column was used in combination with the mobile phase tetrahydrofuran - water - acetonitrile (55:8:37)(Fig.10.8)¹⁰.

Morris and Lee⁷ analyzed potato alkaloids on octyl and octadecyl-type stationary phases. Using a mobile phase consisting of acetonitrile - water that contained small amounts of ethanolamine (less than 0.1%), detection at 200 nm was possible. The separation of α -chaconine and α -solanine could be achieved on an octadecyl column with acetonitrile - water - ethanolamine (45:55:0.1)(Fig.10.9e) or on an octyl column with the same solvent in the ratio (55:45:0.1)(Fig.10.9f). The alkaloids could also be separated on silica gel with this mobile phase in the ratio (77.5:22.5:0.05)(Fig.10.9g). In the case when solanidine was present in the extracts, the silica gel column was preferred. Hydrolysates of the α -chaconine and α -solanine could also be analyzed with the octadecyl column (Fig.10.9a-d). The systems could also be used for the analysis of potato extracts (Fig.10.9e-g). For a total glycoalkaloid analysis, the normal-phase system gave the fastest results (Fig.10.9h).

10.2. DETECTION

Most of the steroidal alkaloids have UV absorption only at about 200 nm. Using UV detectors with variable wavelength, it is possible to analyze microgram amounts of such alkaloids.

For further identification of the separated steroid alkaloids, Bushway et al.^{2,5} used UV absorption ratios at 215, 225, 235 and 245 nm. For the detection, a wavelength of 215 nm was preferred to 208 nm because of a more stable baseline, and with sufficient sensitivity. The sensitivity could be increased by using acetonitrile - water as mobile phase. Such a solvent system allowed detection at 200 nm.

Morris and Lee⁷ detected the potato glycoalkaloids at 200 nm, which they found to be 960% more sensitive than detection at 215 nm and 55% more sensitive than detection at 205 nm; the detection limit was 0.1 μ g, which also compared favorably with RI detection (detection limit 5 μ g). At 200 nm there was no interference of sugars as was observed at 195 nm. The ethanolamine in the mobile phase had to be kept below 0.1%, otherwise the background absorption of the mobile phase became too high.

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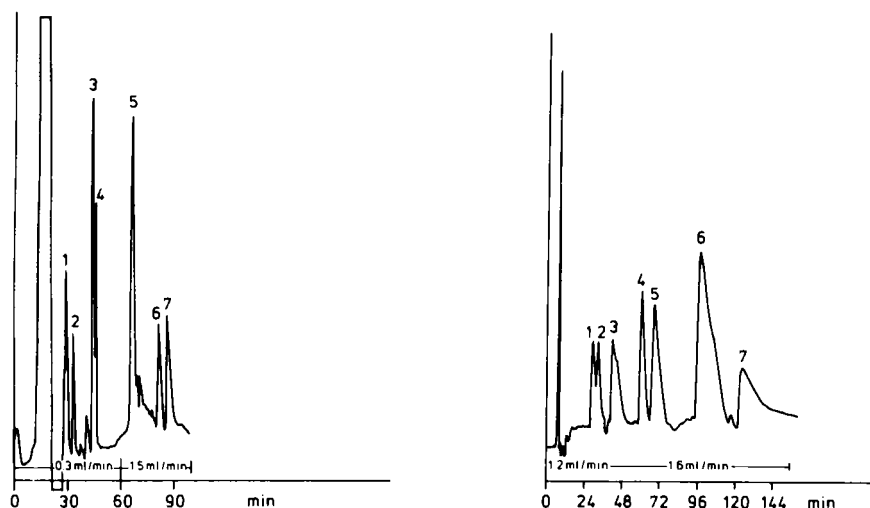


Fig. 10.1. Separation of some steroidal alkaloids⁴
Column Zorbax-Sil 6 μ m (500x4.6 mm ID), mobile phase *n*-hexane - methanol - acetone (18:1:1), flow rate 0.3 ml/min (60 min), followed by 1.5 ml/min, detection UV 213 nm. Peaks: 1, tomatillidine; 2, solanidine; 3, tomatidine; 4, 5-tomatidenol; 5, solasodine; 6, veramine; 7, rubijervine.

Fig. 10.2. Separation of some steroidal alkaloids⁴
Column Zorbax-Sil 6 μ m (500x4.6 mm ID), mobile phase *n*-hexane - ethanol - acetone (18:1:1), flow rate 1.0 ml/min (36 min), followed by 1.6 ml/min, detection UV 213 nm. Peaks: 1, isorubijervine; 2, rubijervine; 3, muldamine; 4, veratramine; 5, verarine; 6, cyclopamine; 7, jervine.

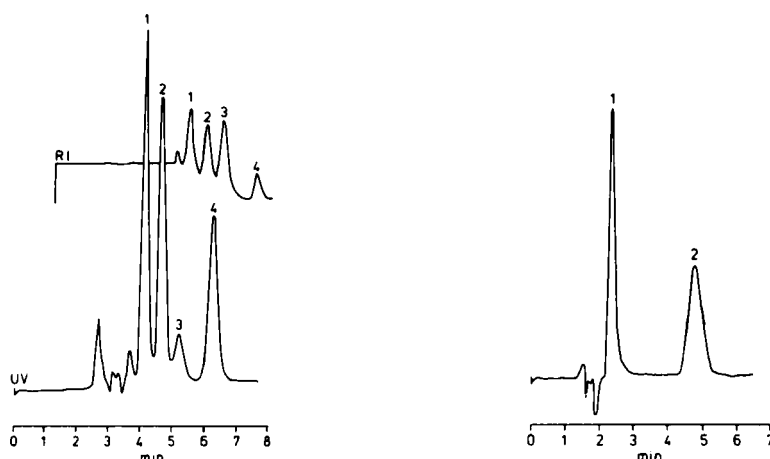


Fig. 10.3. Separation of some glycoalkaloids²
Column μ Bondapak NH₂ (300x4 mm ID), mobile phase tetrahydrofuran - 0.025 M potassium dihydrogen phosphate - acetonitrile (2:1:1), flow rate 1 ml/min, detection refractometer and UV 208 nm. Peaks: 1, β -chaconine; 2, α -chaconine; 3, tomatine; 4, α -solanine.

Fig. 10.4. Separation of glycoalkaloids present in dried potato peels²
Column μ Bondapak Carbohydrate (300x4 mm ID), mobile phase tetrahydrofuran - water - acetonitrile (56:14:30), flow rate 2 ml/min, detection UV 215 nm. Peaks: 1, α -chaconine; 2, α -solanine.

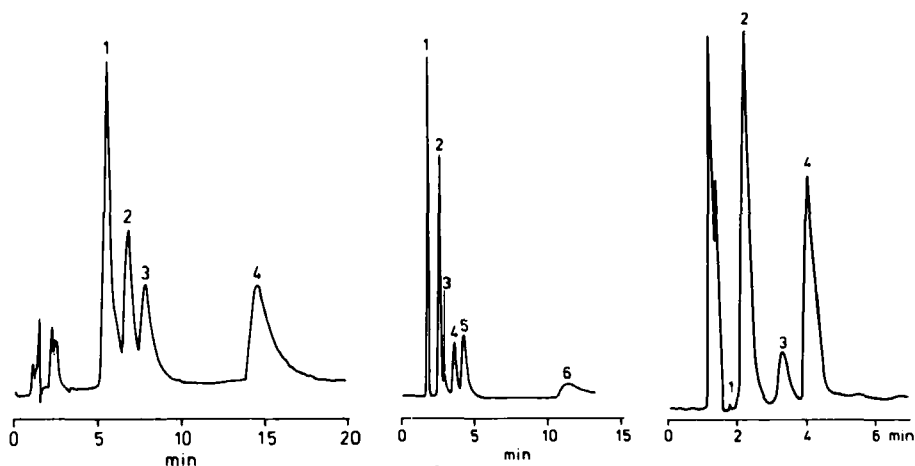


Fig. 10.5. Separation solasodine glycosides³
Column μ Bondapak C18 (300x3.9 mm ID), mobile phase methanol - 0.01 M Tris buffer (3:1), flow rate 2 ml/min, detection UV 205 nm. Peaks: 1, α -glycosides; 2, β -glycosides; 3, γ -glycosides; 4, solasodine.

Fig. 10.6. Separation of solasodine glycosides³
Column μ Bondapak Carbohydrate (300x3.9 mm ID), mobile phase isopropanol - methanol (7:3), flow rate 2 ml/min, temperature 40°C, detection UV 205 nm. Peaks: 1, solasodine and solasodiene; 2, β - and γ -solamargine; 3, γ -solasonine; 4, α -solamargine; 5, β -solasonine; 6, α -solasonine.

Fig. 10.7. Separation of glycoalkaloids from a potato meal extract⁸
Column Radial-Pak μ Bondapak NH₂ (100x8 mm ID), mobile phase tetrahydrofuran - acetonitrile - water - methanol (55:30:10:5), flow rate 3.0 ml/min, detection UV 215 nm. Peaks: 1, γ -chaconine; 2, β_2 -chaconine; 3, β_1 -chaconine; 4, α -chaconine.

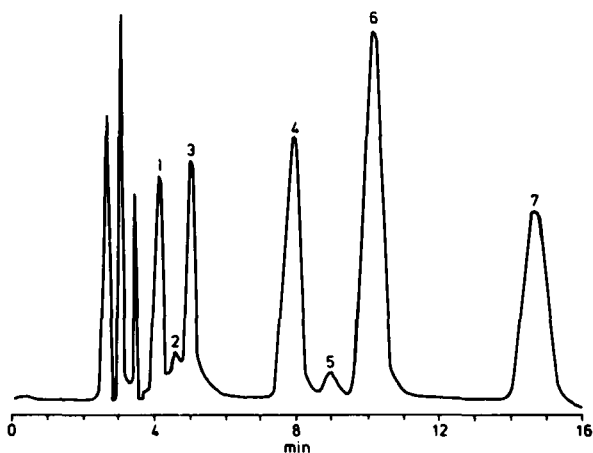


Fig. 10.8. Separation of metabolites of α -chaconine and α -solanine¹⁰
Column μ Bondapak Carbohydrate (300x4 mm ID), mobile phase tetrahydrofuran - water - acetonitrile (55:8:37), flow rate 1.1 ml/min, detection UV 215 nm. Peaks: 1, γ -chaconine; 2, γ -solanine; 3, β_2 -chaconine; 4, β_1 -chaconine; 5, unknown; 6, α -chaconine; 7, β_2 -solanine. (reproduced with permission from ref. 10, by the courtesy of Marcel Dekker Inc.)

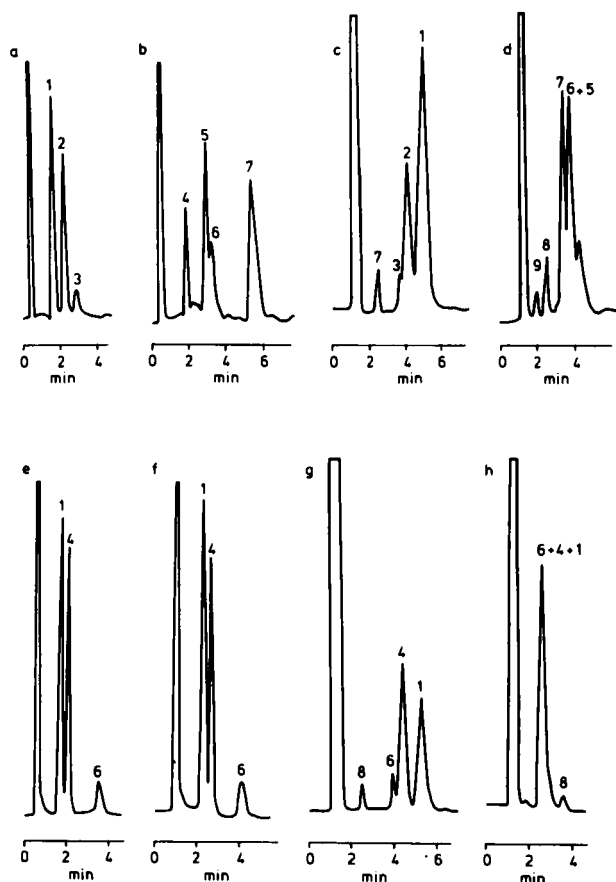


Fig. 10.9. Separation of glyco-alkaloids of the α -chaconine and α -solanine series⁷
 Chromatogram a, b and e: column Radial-Pak C18 (100x8 mm ID), mobile phase acetonitrile - water - ethanolamine (45:55:0.1), flow rate 3 ml/min, detection UV 200 nm.
 Chromatogram c, d, g and h: column Radial-Pak Silica (100x8 mm ID), mobile phase acetonitrile - water - ethanolamine (775:225:0.5), flow rate 3 ml/min, detection UV 200 nm.
 Chromatogram f: column Radial-Pak C8 (100x8 mm ID), mobile phase acetonitrile - water - ethanolamine (55:45:0.1), flow rate 3 ml/min, detection UV 200 nm.
 Peaks: 1, α -solanine; 2, β -solanine; 3, γ -solanine; 4, α -chaconine; 5, β_1 -chaconine; 6, β_2 -chaconine; 7, γ -chaconine; 8, solanidine; 9, solanadiene.

TABLE 10.1

HPLC ANALYSIS STEROIDAL ALKALOIDS

ALKALOIDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID (mm)	MOBILE PHASE	REF.
Tomatidine,solanidine,solasodine,rubijervine,veratramine,jervine	Preparative HPLC	Porasil A 37-75 μm	2440x12 OD	gradient elution A. Me_2CO -n-hexane(2:1) B. 97% aq. Me_2CO	1
α - and β -chaconine, α -solanine,tomatine	Analysis potato alkaloids (Fig.10.3 and 10.4)	$\mu\text{Bondapak C18}$ $\mu\text{Bondapak NH}_2$ $\mu\text{Bondapak Carbohydrate}$	300x4 300x4 300x4	THF- H_2O -ACN(5:3:2) THF-0.025M KH_2PO_4 -ACN(2:1:1),(5:3:2) THF- H_2O -ACN(56:14:30),(6:1:3) ACN- H_2O (85:15)	2
Solasodine,solasodine glycosides,solasodiene	Analysis plant material and hydrolyzed glycosides (Fig. 10.5 and 10.6)	$\mu\text{Bondapak C18}$ $\mu\text{Bondapak Carbohydrate}$	300x4 300x4	MeOH-0.01M Tris buffer(88:12),(3:1) ACN-0.01M Tris buffer(7:3),(2:3) MeOH-isoprOH(3:7) IsoprOH-cyclohexane(4:1)	3
14 <i>Solanum</i> and <i>Veratrum</i> alkaloids	Separation steroidal alkaloids(Fig.10.1 and 10.2)	Zorbax Sil 6 μm	250x4.6	n-Hexane-MeOH- Me_2CO (18:1:1) n-Hexane-EtOH- Me_2CO (18:1:1)	4
α -Chaconine, α -solanine	Quantitative analysis potato alkaloids	$\mu\text{Bondapak Carbohydrate}$	300x4	THF- H_2O -ACN(53:17:30)	5
Solasodine,various steroids	Analysis in <i>Solanum</i> species	Zorbax Sil 6 μm	300x4.6	n-Hexane-MeOH- Me_2CO (18:1:1)	6
α -, β_1 -, β_2 - and γ -chaconine, α -, β - and γ -solanine,solanidine,solanadiene	Analysis potato alkaloids (Fig.10.9)	Radial-Pak C8 Radial-Pak C18 Radial-Pak Silica	100x8 100x8 100x8	ACN- H_2O -ethanolamine(50:50:0.2), (55:45:0.1) Idem (36:65:0.2),(45:55:0.1) Idem (77.5:22.5:0.5),(45:55:0.01)	7
α -, β_1 -, β_2 - and γ -chaconine, α -, β - and γ -solanine	Analysis potato alkaloids (Fig.10.7)	Radial-Pak NH_2	100x8	THF-ACN- H_2O -MeOH(55:30:10:5), (50:25:15:5:9.5)	8
α -Chaconine, α -solanine,commer-sonine,demissine	Semipreparative HPLC potato glyco-alkaloids	Zorbax NH_2	250x9.4	THF- H_2O -ACN((55:20:25)	9
α -, β_1 -, β_2 - and γ -chaconine, α -, β_1 -, β_2 - and γ -solanine	Determination metabolites of potato glyco-alkaloids(Fig. 10.8)	$\mu\text{Bondapak Carbohydrate}$	300x4	THF- H_2O -ACN(55:8:37)	10

Chapter 11

XANTHINE ALKALOIDS

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The analytical problems concerning xanthine derivatives can be grouped as follows:

1. Analysis of theophylline in biological fluids (Table 11.8).
2. Analysis of pharmaceutical preparations containing caffeine (Table 11.7).
3. Analysis of drugs of abuse containing caffeine as adulterant (Table 11.5).
4. Analysis of caffeine, theobromine and theophylline in food and beverages (Table 11.9).

11.1 THEOPHYLLINE IN BIOLOGICAL FLUIDS

In the numerous studies on the analysis of theophylline in biological fluids, only a few HPLC systems have been used. However, a variety of sampling techniques have been described, ranging from direct injection of plasma to solvent extraction techniques. These techniques will be discussed briefly later (see below). Whereas series of drugs has been reported not to interfere with the analysis of theophylline^{18,27,33,35,48,68,75,96,104,105,106,111,112,122,126,127,128,156,160,183,191,193,196}, some drugs do interfere, e.g. ampicillin and methicillin^{54,140,187}, cephalosporins^{77,81,128,137}, acetazolamide^{82,101}, sulfadiazine¹⁰², procainamide¹⁷⁶, and sulfamethoxazole^{128,139,140}. The interference of caffeine metabolites (theophylline, theobromine, 1,7-dimethylxanthine (paraxanthine), 3-methylxanthine, 7-methylxanthine, 1-methyluric acid and 1,3-dimethyluric acid)^{51,63,67,144,165,177,185,186,196}, theophylline metabolites and other xanthine metabolites has been dealt with in a number of studies^{56,63,76,80,95,96,98,106,112,116,128,129}. The xanthine derivative dyphylline has also been reported to interfere with the analysis of theophylline^{183,187}.

For the quantitation of theophylline, an internal standard has in most cases been used, e.g. 8-chloro-theophylline, β -hydroxyethyl-theophylline and β -hydroxypropyl-theophylline.

Several authors have noted peak anomalies for theophylline in the case when samples dissolved in acetonitrile (used for deproteinization) were analyzed on an octadecyl type of column^{112,159,196}.

For direct analysis of theophylline in plasma and serum, the use of pre-columns has been recommended in order to prolong column life^{18,63,91,99,109,111,122,138,146,177,192,196}. Bates and Bernstein³⁴ reported a method in which no pre-column was necessary.

Comparison of HPLC with GLC^{27,53,98,100,104,113,128,129}, UV spectrometry^{26,27,33,35,59,100,128,154}, EMI^{69,72,97,100,110,127,135,154,157,196}, FIA¹⁷⁹ and RIA^{154,155,165} has been made, regarding their applicability to the analysis of theophylline in biological fluids. Because of interference in UV spectrometry, HPLC was preferred by all authors. The time-consuming

preparation of samples necessary for GLC, makes HPLC preferable. The results of EMIT and HPLC had generally good correlation^{69,72,100,127,136,157,196}. However, Sheen et al.⁹⁷ and Tin et al.¹¹⁰ reported that significantly higher values were found with EMIT. HPLC was found to be more precise, but EMIT had the advantage of shorter analysis time, and the analysis was easier to perform. Reviews of theophylline monitoring have been given^{50,78,83,132,188}.

11.2. SAMPLE PREPARATION FOR XANTHINE ASSAYS IN BIOLOGICAL FLUIDS

For the assay of theophylline in biological fluids the following types of sample preparation have been described:

1. Direct injection of plasma, serum or saliva.
2. Deproteinization of plasma, serum or saliva prior to injection.
3. Solvent extraction of the drug from biological fluids prior to injection.
4. Preconcentration of the drug on adsorbent columns.

1. The first method has the disadvantage of very short column life due to contamination with plasma proteins⁷². By using precolumns, which were replaced every 30-40 samples, the problem could be solved^{16,91}. Saliva samples could be injected directly without problems²⁶. Manno et al.¹¹¹ injected serum, plasma and saliva directly on the column. By regularly cleaning of the column by subsequently pumping water, methanol - water (1:1) and methanol through the column, it could be used for 400-500 samples. Yoshida et al.¹⁸¹ treated an octadecyl type column with human plasma. This column could then be used for drug monitoring by direct injection of plasma samples, giving good reproducibility and complete recovery of the drugs.

2. Several methods have been described to remove the proteins from the sample. Addition of trichloroacetic acid^{26,68,72,120,147} or perchloric acid⁶⁵ followed by centrifuging has been reported, as well as addition of organic solvents, e.g. acetonitrile^{55,56,76,80,86,105,122,138,140,176,177,191,196} and methanol^{95,104} in which the internal standard has been dissolved, also followed by centrifuging, whereafter the clear supernatant was used for injection.

Deproteinization can also be achieved by molecular filtration^{27,63,97}. Desiraju et al.⁶³ preferred such filtration above addition of trichloroacetic acid, acetone, acetonitrile or ammonium sulfate. The former two additives caused interfering peaks, the latter two were not effective. Van den Bemd et al.⁹⁵ found filtration and the addition of methanol were compatible. Bateman et al.⁷⁴ used ultrafiltration of saliva prior to direct injection.

3. The most widely used procedure is extraction of the drug from the biological material by means of an organic solvent at various pH. Chloroform - isopropanol (95:5) has been successfully used to extract theophylline from plasma, serum and saliva samples. The samples were either extracted directly^{26,35,54,98,112,129,156,160,187} or after the addition of an acid^{14,30,72,95,99}, ammonium sulfate^{19,51,67,110,156,165,168} or buffers: pH 6.5^{33,127} or pH 7.4⁴⁴. Extraction at pH 8.6, with chloroform - isopropanol (9:1), avoids interference of acetazolamide in the HPLC analysis¹⁰¹. Theophylline was extracted from brain homogenates with chloroform at pH 7¹¹³. Dyphylline was extracted from serum or plasma with chloroform - isopropanol (9:1) after the addition of sodium hydroxide¹²⁵.

Other organic solvents used for the extraction of theophylline and other xanthine derivatives are: dichloromethane - diethyl ether (4:7)^{71,96}; dichloromethane - isopropanol (4:1)¹⁰²; dichloromethane - methanol (9:1) or dichloromethane alone, after the addition of dilute hydrochloric acid^{126,136}; dichloromethane¹⁴⁴; sec-butanol - n-hexane (2:3) after the addition of

ammonium sulphate⁴⁸; *t*-pentanol - chloroform - 0.1% hydrochloric acid (80:19.9:0.1)⁷⁵; ethyl acetate^{49,183}; and *t*-pentanol - ethylene chloride at pH 6.9⁷⁹. Ion-pair extraction with tetrabutylammonium as counter-ion at pH 6 enabled the extraction in one step of both theophylline and uric acid derivatives (for the analysis of theophylline and its metabolites in urine¹⁴⁶). A similar method (pH 11.0) has been used for caffeine and its metabolites¹⁹². Jusko and Poliszczuk²⁶ compared an extraction and a deproteinization procedure and found that extraction was more sensitive than deproteinization with trichloroacetic acid²⁶ or acetonitrile⁷⁹. Soldin and Hill⁵⁴ preferred extraction to direct serum injection because of the possible interference of ampicillin and methicillin in the latter case. Interference of cephalosporins can be resolved by using an extraction step prior to HPLC⁷⁷. Extraction yields cleaner samples than deproteinization, but is more time consuming⁷².

4. Concentration of the drug on adsorbent columns has been done for the analysis of theophylline and its metabolites in urine¹⁶ and for some other xanthine derivatives in urine and plasma⁶². Thompson et al.¹⁶ used an anion-exchange resin for pre-fractionation in a xanthine and uric acid fraction. Bye and Brown⁶² collected the xanthine derivatives on a metal-chelate resin column.

The solvent used for the injection of theophylline plasma samples was shown to affect critically the chromatographic results^{112,129}. When the mobile phase was used as solvent (acetonitrile - sodium acetate buffer (8:92)) the best results were obtained. Pure acetonitrile resulted in poor peak shape. Jowett¹⁵⁹ found that acetonitrile used during deproteinization had to be removed prior to HPLC analysis, otherwise extra peaks were observed in the chromatogram of theophylline. Muir et al.¹⁹⁶ found that the complete removal of acetonitrile prior to the HPLC analysis was essential. Mann¹⁰³ found that the pH of the injected sample influenced the retention times of theophylline and 8-chlorotheophylline. By adding acetic acid to the sample prior to injection, variations in the retention times of the alkaloids in the serum samples could be avoided.

The extraction methods can generally be regarded as most sensitive and specific. The deproteinization methods require regular cleaning of the column and the use of replaceable pre-columns; but they are more simple and rapid than the extraction methods.

11.3. ION-EXCHANGE HPLC

For the analysis of theophylline and its metabolites in biological fluids, an Aminex A-5 cation-exchange resin has been used in combination with a mobile phase consisting of 0.45 M ammonium dihydrogen phosphate buffer (pH 3.65)(Table 11.1)^{16,18,97}.

Jusko and Poliszczuk²⁶ reported the analysis of theophylline in biological fluids by using a chemically bonded strong cation-exchanger on pellicular beads (Table 11.2). 0.66% Aqueous acetic acid was used as mobile phase. The same mobile phase was applied in combination with a microparticulate chemically bonded strong cation-exchanger by Peng et al.⁴⁹.

Walton et al.¹²⁰ separated xanthines on a 4% crosslinked cation-exchange resin. The effect of the counter-ion of the cation-exchanger, the pH, and the percentage of organic solvent in the aqueous mobile phase, was studied - and the analysis of xanthines in serum and beverages was described.

Analgesics, including some xanthines, have been analyzed by ion-exchange chromatography (ref. 1,2,3,6,8,10,12,13,15,29,38,89). Murgia et al.¹⁰, Walton¹⁵, Murgia³⁸ and Hanai et al.⁸⁹

TABLE 11.1

SEPARATION OF SOME URIC ACID AND XANTHINE DERIVATIVES¹⁶

Column Aminex A-5 cation-exchange resin, 13 μ m (665x3.2 mm ID), mobile phase 0.45M ammonium dihydrogen phosphate buffer (pH 3.65), flow rate 10 ml/hr, temperature 55°C, detection UV 280 nm.

Compound	Retention time(min)	Compound	Retention time(min)
Uric acid	10.2 min	Theobromine	17.0
3-Methyluric acid	11.3	1-Methylxanthine	17.3
Xanthine	13.3	Hypoxanthine	19.2
1-Methyluric acid	13.7	Theophylline	20.0
3-Methylxanthine	14.8	Paraxanthine	22.8
7-Methylxanthine	15.2	Caffeine	26.0
1,3-Dimethyluric acid	15.6		

TABLE 11.2

RELATIVE RETENTION OF VARIOUS XANTHINE DERIVATIVES RELATED TO THEOPHYLLINE²⁶

Column Zipax SCX (1000x2.1 mm ID), mobile phase 0.66% aqueous acetic acid, detection UV 254 and 280 nm, column pressure 1200 psi.

Compound	Relative retention	Compound	Relative retention
Theophylline	1.00	Uric acid	0.76
Caffeine	1.66	1-Methyluric acid	0.76
Theobromine	0.97	1,3-Dimethyluric acid	0.80
1-Methylxanthine	0.97	8-Nitrotheophylline	0.90
3-Methylxanthine	0.79	8-Chlorotheophylline	0.90
Xanthine	0.79	8-Bromotheophylline	0.90
Hypoxanthine	1.59	Dihydroxypropyltheophylline	0.86
Paraxanthine	1.76	8-Hydroxypropyltheophylline	1.41
8-Chloroxanthine	0.79	Phenobarbital	0.68

tested different ion-exchange resins for the separation of analgesics. The influence of the counter-ion, the pH, the degree of crosslinking, and solvent composition on the separation, were investigated. It was concluded that no ion-exchange was involved, but that the dominant mechanism of retention was adsorption on the resin matrix. The best results were obtained with ammonium or chloride counter-ions for, respectively, cation- and anion-exchange resins. Non-ionogenic resins were also tested.

Ligand-exchange chromatography was used by Wolford et al.³ to separate oxypurines. Walton¹⁵ has given a review on ligand-exchange and matrix-affinity chromatography.

11.4. REVERSED-PHASE HPLC

For the analysis of theophylline in biological fluids, a reversed-phase separation on a microparticulate stationary phase with chemically bonded octadecyl groups, and a mobile phase consisting of a 0.01 M sodium acetate buffer (pH 4.0) containing 4-10% acetonitrile, is widely used (Table 11.3 and Fig.11.1)²⁷.

The reversed-phase system allows the direct injection of deproteinized serum or plasma samples, whereby the analysis time is considerably reduced. However, under these conditions, a number of other drugs, e.g. ampicillin, methicillin⁵⁴, cephalosporins^{77,137}, acetazolamide (ref. 82,101) and trisulfapyrimidines¹⁰² interfered with the analysis of theophylline in the

TABLE 11.3

SEPARATION OF SOME XANTHINE DERIVATIVES^{54,112,138,158}

- S1: Column μ Bondapak C18 (300x4 mm ID), mobile phase acetonitrile - 0.02M sodium acetate buffer (pH 4.0)(1:9), flow rate 1.8 ml/min, detection UV 254 nm⁵⁴.
 S2: Column Hypersil ODS 5 μ m (100x5 mm ID), mobile phase acetonitrile - 0.02M sodium acetate buffer (pH 4.0)(8:92), flow rate 1.5 ml/min, detection UV 273 nm¹¹².
 S3: Column Spherisorb ODS 10 μ m (250x4.6 mm ID), mobile phase acetonitrile - 0.01M sodium acetate buffer (pH 4.0)(15:85), flow rate 1.0 ml/min, detection UV 273 nm¹³⁸.
 S4: As system S3, solvent ratio(18:82)¹³⁸.
 S5: Column Ultrasphere ODS 5 μ m (150x4.6 mm ID), mobile phase acetonitrile - 0.01M sodium acetate buffer (pH 6.5)(9:91), flow rate 1 ml/min, detection UV 273 nm¹⁵⁸.

Compound	S1	S2	S3	S4	S5
Uric acid	1.78		2.88	2.79	1.03
Xanthine	2.00	1.00	3.04	3.05	1.35
Hypoxanthine	2.00	0.95	3.08	3.10	1.32
3-Methyluric acid	2.00	0.95			
7-Methyluric acid			3.45	3.07	1.06
1-Methyluric acid	2.37	1.15	3.51	3.13	1.12
3-Methylxanthine	2.65	1.35	3.66	3.59	1.84
7-Methylxanthine			3.97	3.77	1.75
1-Methylxanthine	2.93	1.40	4.26	3.82	2.00
1,3-Dimethyluric acid	3.23	1.65	3.83	3.75	1.47
Theobromine	3.65	1.65	4.78	4.47	2.60
Theophylline	4.88	2.50	5.76	5.14	3.85
Paraxanthine		2.50	5.96	5.31	3.85
Caffeine	8.47	4.65	10.08	8.33	7.53
β -Hydroxypropyltheophylline	8.90		7.98	6.60	9.02
β -Hydroxyethyltheophylline		3.20			
Paracetamol		2.15			
Phenobarbital		29.0			

method described. Extraction of theophylline from the biological material to be analyzed eliminated the interference of the compounds mentioned. Also, a caffeine metabolite, 1,7-dimethylxanthine, can interfere with the theophylline analysis⁹⁵. Some studies reported no significant amounts of this metabolite are to be found in the plasma or serum^{98,112,129}, whereas others reported considerable amounts present, and causing errors of up to 30% (in the case where no separation was obtained between theophylline and the metabolite^{185,186,196}).

By the addition of tetrahydrofuran to the above mentioned mobile phase and by increasing the pH of the buffer to 5 the interference of 1,7-dimethylxanthine could be obviated^{106,122}. The same was the case with the other drugs mentioned above (ampicillin, etc.). Also, by decreasing the percentage of acetonitrile in the mobile phase, the interference of ampicillin could be eliminated¹¹¹. With ageing of the columns, Marion et al.¹⁷⁶ found that procainamide interfered with theophylline.

The pH of the buffer is important for the separation. The retention of 8-chlorotheophylline in particular is greatly dependent on the pH, and it can be varied at will by changing the pH^{35,112,129}.

Also, for the analysis of caffeine, the sodium acetate buffer containing acetonitrile (as mentioned above) has been applied^{136,138}. It has also been used for dyphylline assays in biological fluids^{57,102,123,147}. Due to the interference of benzoic acid (among others present in caffeine injections) Blanchard et al.¹⁵⁸ modified an earlier reported method¹³⁸ (Table 11.3). Changing the pH of the acetate buffer to 6.5 and decreasing the amount of acetonitrile in the mobile phase lead to a separation of benzoic acid and caffeine. The separation of the caffeine metabolites in this system is, however, not as good as under the conditions original-

ly reported.

A gradient of acetonitrile - aqueous acetic acid has been used in connection with an octadecyl column for assays of caffeine and its metabolites in biological fluids^{107,133}. The system has also been used to eliminate the interference of sulfamethoxazole in the analysis of theophylline in serum¹³⁹.

George and Patel²⁰⁸ investigated some reversed-phase column packing materials for the presence of active silanol groups, by using the columns in the adsorption mode with *n*-heptane as solvent. Four materials were tested for the analysis of theophylline and caffeine. These two compounds were well separated on all materials with the mobile phase acetonitrile - water - acetic acid (95:5:0.2). However, the sequence of caffeine and the internal standard β -hydroxypropyltheophylline varied for the columns.

Broussard¹⁶⁰ reported the analysis of theophylline in serum using a modified method of Adams et al.³⁵. As mobile phase, water - methanol - acetonitrile - 10 ml/l acetic acid (788:180:16:16) was used in combination with an octadecyl type of column. A series of non-interfering drugs was reported. According to Jonkman et al.^{185,186} 1,7-dimethylxanthine co-elutes with theophylline in this system and causes errors of up to 30% (see also ref 196, Fig.11.8).

Phosphate buffers has been used in place of acetate buffers in some cases^{55,80,103,152,178,181,183,187,197} (Fig.11.2). Volatile buffers have been used to avoid corrosion¹⁵⁷. Non-buffered mixtures of water and acetonitrile have given good results in the analysis of theophylline (ref. 76,113,161) and in the analysis of dyphylline¹³⁰ in biological fluids.

To avoid the toxic acetonitrile, methanol has been preferred. Peat et al.⁵⁹ analyzed theophylline on an octadecyl column with methanol - 0.01 M sodium acetate buffer (1:3). In this case too the pH of the buffer had great influence on the retention of 8-chlorotheophylline. The optimum pH for the analysis was found to be 4. Similar systems were found to be useful for eliminating the interference of such drugs as ampicillin, methicillin, etc. (mentioned above)^{104,140,191}.

Phosphate buffer (pH 4.7) - methanol (88:12) was used in the analysis of theophylline and its metabolites on an octadecyl column (Fig.11.3)⁶³. Anderson and Murphy⁴⁶ used a buffer of pH 6.0 in 25% methanol to obtain a separation of some xanthines. Miller and Tucker¹⁰⁹ preferred a phosphate buffer of pH 2.3 in 40% methanol for the serum analysis of a series of drugs - including some xanthine derivatives. Several other related systems have been applied for the analysis of theophylline^{65,74,91,99}. For the automated HPLC analysis of theophylline in serum, methanol - 0.0025 M sodium dihydrogen phosphate (14:86) containing 0.065% triethylamine (pH 6.6) has been used in combination with an octyl type of stationary phase¹⁹³. Two hundred drugs were tested for interference in this system.

To separate theophylline from 1,7-dimethylxanthine, Rodriguez et al.¹⁷⁷ used methanol - formamide - potassium dihydrogen phosphate (0.05 M)(22:11.5:66.6)(pH 5.8) in combination with an octadecyl type of column.

The already mentioned influence of the pH of the buffer on the retention of 8-chlorotheophylline was investigated by Hill⁶⁴, who found that an optimum separation of xanthine derivatives was obtained at a pH of 5.0 for a mobile phase consisting of 1% propionic acid - methanol (8:2) on an octadecyl column. For a simultaneous analysis of theophylline, proxiphylline and other xanthine derivatives in serum, Nielsen Kudsk and Kirstein Pedersen⁶⁸ found that acetonitrile - acetate buffers were unsatisfactory, and preferred methanol - 0.02 M potassium chloride (pH 2) (3:7) as mobile phase in connection with an octadecyl column (Fig.11.4).

Weidner et al.¹²⁸, on the other hand, performed analyses of theophylline on an octadecyl column with isopropanol - 0.1 M phosphate buffer (pH 3.8)(4:96)(Fig.11.5). They extensively discussed the interference of other drugs in the analysis.

Rosenbaum¹³ analyzed analgesics by means of microparticulate octadecyl columns, because he found that the columns had a longer life under the conditions used for the analysis than did ion-exchange columns.

Jandera et al.¹¹⁸ studied the influence of various gradient systems of methanol - water, in combination with octadecyl columns, on the retention behaviour of xanthines, while isocratic elution with such solvents was applied for the analysis of crude drugs containing xanthine derivatives¹³¹.

The peak broadening caused by the sample solvent in the analysis of analgesics was investigated by Williams et al.¹⁴⁵. For reversed-phase systems, the best results were obtained with solvents that had about the same polarity as the mobile phase used (Fig.11.6).

For the analysis of xanthine derivatives in food and beverages, octadecyl type of columns have been used in combination with the mobile phase methanol - water - acetic acid (20:79:1) (ref. 86,141,148), or similar ratios^{142,205,207}.

Jonkman et al.¹⁵⁶ reported the use of a non-polar mobile phase (chloroform - heptane - absolute ethanol - water - acetic acid (600:400:32:1.5:0.8)) in combination with an octadecyl type of stationary phase. The advantage of this method is that chloroform - isopropanol extracts of serum can be injected directly on the column.

To optimize a chromatographic assay, Weyland et al.²⁰² reported the application of an operational research technique called non-linear programming.

11.5. ION-PAIR HPLC

Muir et al.¹⁴⁶ determined theophylline and its metabolites in urine by ion-pair HPLC. The method was based on a combination of liquid-liquid and ion-pair liquid-liquid extraction, followed by reversed-phase ion-pair separation. It allowed simultaneous analysis of xanthine and uric acid derivatives. The method was further improved for the analysis of caffeine and its metabolites (Fig.11.7)¹⁹². It was also modified for the analysis of theophylline in plasma and saliva¹⁹⁶. This method had the advantage that the interference of the caffeine metabolite, 1,7-dimethylxanthine (paraxanthine), was avoided (Fig.11.8). The authors reported that a typical number of cups of coffee may result in apparent plasma theophylline concentrations of ca 3 mg/l, if the 1,7-dimethylxanthine is not separated from theophylline.

Methods previously described for the analysis of theophylline in urine^{63,108} were found to be less suitable because of interfering peaks, even in blank urine. In a study on ion-pair chromatography of multicomponent drugs, Huen et al.⁸⁸ included caffeine (see Chapter 4).

Because of their weak basic properties, the xanthine derivatives do not form ion-pairs with alkylsulfonates, and are thus little affected by changes in the concentration or in the alkyl-chain length of the pairing-ions^{169,170,171}.

11.6. STRAIGHT-PHASE HPLC

Sitar et al.¹⁹ used straight-phase HPLC to analyse theophylline in biological fluids. A microparticulate silica gel column and a mobile phase consisting of chloroform - isopropanol -

TABLE 11.4

STRAIGHT-PHASE HPLC SEPARATION OF SOME XANTHINE DERIVATIVES^{51,53,67}

Column Lichrosorb Si60, 5 μ m (250x3 mm ID), mobile phase dichloromethane - methanol containing 0.02g ammonium formate and 0.017ml 97% formic acid per 100ml (978:22), flow rate 2ml/min, detection UV 280 nm.

Compound	Retention time(min)	Compound	Retention time(min)
Theobromine	2.4	3-Methylxanthine	7.4
Theophylline	2.9	7-Methylxanthine	8.4
Paraxanthine	3.6	1-Methylxanthine	9.8
Prednisolone(internal standard)	4.4		

acetic acid (84:15:1) was used. Similar systems have been described where other ratios of chloroform - methanol - acetic acid have been used^{53,110}. Midha et al.⁵¹ added up to 40% hexane to such a solvent system in order to compensate for variations in column retentivity, keeping the k' of theophylline at about 7-8. As a second solvent system for the analysis of caffeine metabolites, dichloromethane - ammonium formate buffer in methanol was used^{51,53,67} (Table 11.4).

Evenson and Warren³³ developed an assay for theophylline using microparticulate silica gel columns and a mobile phase of water-saturated chloroform - heptane - acetic acid (300:200:0.4) to which 6% ethanol was added. Heptane was necessary to achieve a separation of theobromine and theophylline.

Boeckx et al.¹²⁷ found that the advantage of the assay mentioned, over methods based on reversed-phase separations, was that silica gel columns used in combination with a simple extraction had a longer life than reversed-phase columns used in combination with a direct injection of deproteinized samples. To avoid the interference of 1,7-dimethylxanthine, a caffeine metabolite, in the analysis of theophylline in biological fluids, Van Aerde et al.¹⁶⁵ developed a separation on silica gel. As the mobile phase, chloroform - dioxane - formic acid (99.5:4.5:0.01) was used.

In addition to acidic solvents, neutral solvents have been used in the analysis of theophylline^{44,48,168} and caffeine¹⁴⁴ in biological fluids - as well as basic solvents^{30,62}. Analgesics have been analyzed on silica gel columns with acidic solvent systems^{25,85}, while xanthine derivatives present in food and beverages have been analyzed on silica gel columns with neutral solvent systems^{24,37} (Fig.11.9). Aigner et al.⁴³ impregnated the silica gel with silver chloride to improve the separation of xanthines.

11.7. DETECTION

The methylxanthine derivatives are usually detected at their absorption maximum - 273 nm - but detection at 254 nm has also been done, although the absorption is much less at this wavelength - for theophylline it is only 35% of that at 273 nm. Peat et al.⁵⁹ used a detector with a fixed wavelength of 280 nm and found it more sensitive than detection at 273 nm. However, other authors reported the opposite¹¹⁶. A disadvantage of detection at 254 nm is the presence of more spurious peaks than at 280 nm¹⁴⁶. With a simultaneous detection at 254 nm and 280 nm van den Bemd et al.⁹⁵ could correct the theophylline plasma levels for interference of 1,7-dimethylxanthine. For identification purpose - so far mainly applied for drugs

of forensic interest - the absorbance ratio at 254 nm and 280 nm has been used, in combination with the relative retention times of the compounds in question¹¹⁵ (see Chapter 2, Table 2.2).

Lewis and Johnson⁸⁰ detected methylxanthines using amperometric methods. Electrochemical detection enabled determination of theophylline in the presence of 1,7-dimethylxanthine, which had the same retention time in the HPLC system employed. As to the sensitivity for theophylline, a pulse amperometric detection compared favorably with UV detection at 254 nm. A combined photometric and amperometric detection offered the advantage of an increased selectivity and the possibility of observing unexpected interference of other compounds in the analysis. Greenberg and Mayer¹¹⁶ also studied electrochemical detection methods for the analysis of theophylline. An increased sensitivity, as well as the possibility of selective attenuation of interference of other compounds, were reported as advantages of electrochemical detection when compared with UV detection.

Hashimoto et al.⁹⁴ developed a capacitance conductivity detector, which was found useful for alkaloid analysis. Caffeine was included in the studies.

Kito et al.¹⁹⁷ reported the selective detection of hypoxanthine and xanthine with an immobilized xanthine oxidase reactor.

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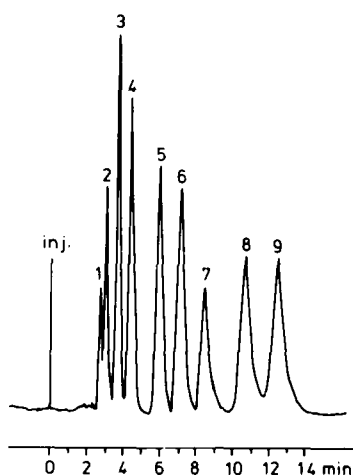


Fig. 11.1. Separation of some xanthine derivatives⁵⁶
 Column μ Bondapak C18 (300x4 mm ID), mobile phase acetonitrile - 0.01 M sodium acetate buffer (pH 4.0) (7:93), flow rate 2.0 ml/min, detection UV 254 nm. Peaks: 1, 1-methyluric acid; 2, 3-methylxanthine; 3, 1,3-dimethyluric acid; 4, theobromine; 5, theophylline; 6, 8-hydroxyethyltheophylline; 7, phenobarbital; 8, caffeine; 9, 8-chlorotheophylline. (reproduced with permission from ref. 56, by the courtesy of Clinical Chemistry)

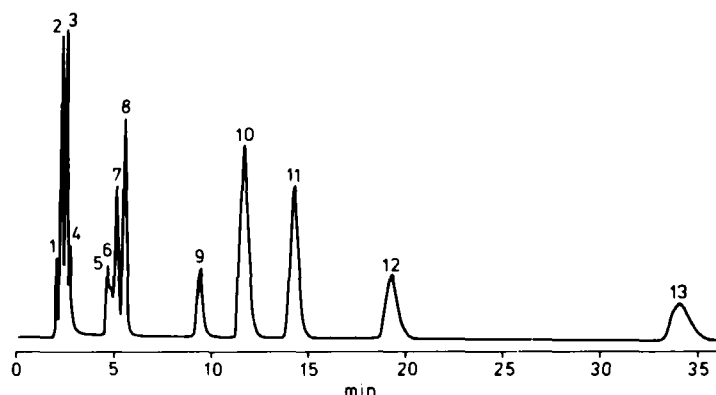


Fig. 11.2. Separation of some xanthine derivatives and urinary metabolites⁸⁰
 Column μ Bondapak C18 (300x4 mm ID), mobile phase acetonitrile - 0.1 M disodium hydrogen phosphate and 0.1 M sodium dihydrogen phosphate in water (2:38), flow rate 1.5 ml/min, detection UV 254 nm. Peaks: 1, uric acid; 2, creatinine; 3, 1-methyluric acid, 3-methyluric acid and 7-methyluric acid; 4, xanthine; 5, 7-methylxanthine; 6, 1,3-dimethyluric acid; 7, 3-methylxanthine; 8, 1-methylxanthine; 9, theobromine; 10, 8-chlorotheophylline; 11, theophylline and 1,7-dimethylxanthine (paraxanthine); 12, dyphylline; 13, caffeine. (reproduced with permission from ref. 80, by courtesy of Clinical Chemistry)

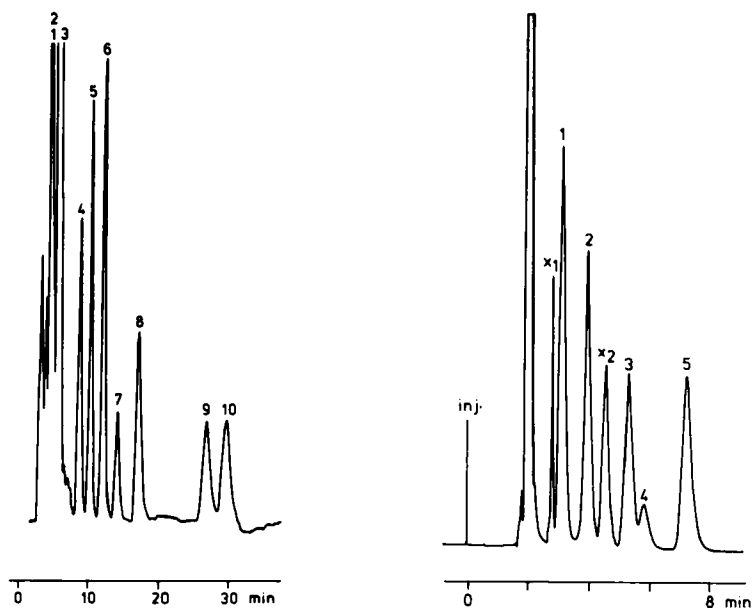


Fig. 11.3. Separation of a standard mixture of xanthine derivatives in serum⁶³
 Precolumn Bondapak C18-Porasil B (40x2.3 mm ID), column μ Bondapak C18 (300x3.9 mm ID), mobile phase methanol - 0.05 M potassium dihydrogen phosphate buffer (pH 4.7) (12:88), flow rate 1.1 ml/min, detection UV 254 nm. Peaks: 1, uric acid; 2, hypoxanthine; 3, xanthine; 4, 1-methyluric acid; 5, 3-methylxanthine; 6, 1-methylxanthine; 7, 1,3-dimethyluric acid; 8, theobromine; 9, theophylline; 10, dyphylline. (reproduced with permission from ref. 63, by the courtesy of Journal Chromatographic Science)

Fig. 11.4. Analysis of theophylline and proxiphylline in serum⁶⁸
 Column μ Bondapak C18 (300x3.9 mm ID), mobile phase methanol - 0.02 M aqueous potassium chloride (pH 2 adjusted with hydrochloric acid)(3:7), flow rate 1.6 ml/min, detection UV 280 nm. Peaks: 1, theobromine; 2, theophylline; 3, proxiphylline; 4, caffeine; 5, 8-chlorotheophylline; x1 and x2 are unidentified compounds constantly present in serum. (reproduced with permission from ref. 68, by courtesy of Acta Pharmacologica et Toxicologica)

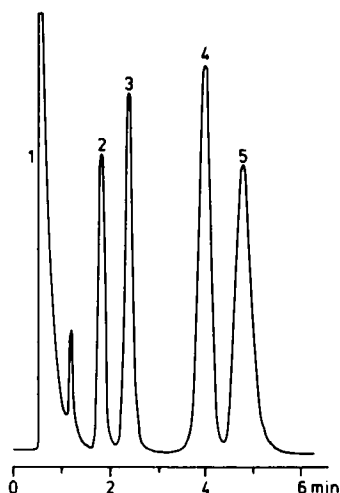


Fig. 11.5. Analysis serum of patient receiving theophylline, caffeine and theobromine are added to the sample¹²⁸
 Column Micropak MCH-10, mobile phase isopropanol - 0.1 M potassium dihydrogen phosphate buffer (pH 3.8, adjusted with phosphoric acid), flow rate 1 ml/min, detection UV 277 nm. Peaks: 1, solvent front; 2, theobromine; 3, theophylline; 4, 8-chlorotheophylline; 5, caffeine. (reproduced with permission from ref. 128, by the courtesy of the American Journal of Clinical Pathology)

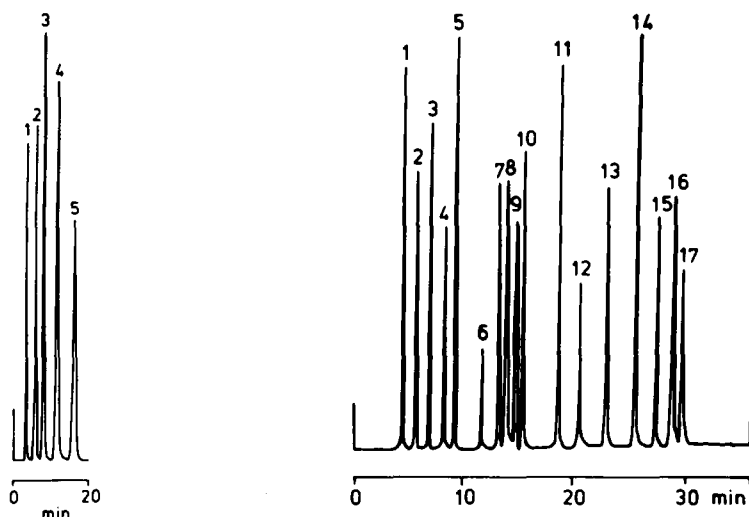


Fig. 11.6. Separation of some analgesics¹⁴⁵
Column Partisil PXS 10/25 ODS2 (250x4.6 mm ID), mobile phase acetonitrile - acetic acid - water (25:5:70), flow rate 1 ml/min, detection UV 275 nm. Peaks: 1, paracetamol (acetaminophen); 2, caffeine; 3, acetylsalicylic acid; 4, salicylic acid; 5, phenacetin.

Fig. 11.7. Separation of some xanthine derivatives¹⁹²
Precolumn Lichrosorb RP2 10 μ m (40x2.1 mm ID), column Ultrasphere ODS 5 μ m (250x4.6 mm ID), mobile phase gradient with solvent A: 0.01 M sodium acetate and 0.005 M tetrabutylammonium hydrogen sulfate in water (pH 4.9), solvent B: same salt concentrations in 50% methanol (pH 4.8). Gradient 0-7.5 min 0% B, 7.5-15 min 0-15% B, 15-25 min 15-30% B, 25-33 min 30-32% B, 33-38 min 32-45% B and 38-41 min 45-0% B. Detection UV 280 nm. Peaks: 1, xanthine; 2, uric acid; 3, 3-methyluric acid; 4, 7-methylxanthine; 5, 3-methylxanthine; 6, 1-methylxanthine; 7, theobromine; 8, 3,7-dimethyluric acid; 9, 7-methyluric acid; 10, 1-methyluric acid; 11, 1,3-dimethyluric acid; 12, 1,7-dimethylxanthine; 13, theophylline; 14, β -hydroxyethyltheophylline (internal standard); 15, 1,7-dimethyluric acid; 16, 1,3,7-trimethyluric acid; 17, caffeine. (reproduced with permission from ref. 192, by the courtesy of Journal Chromatographic Science)

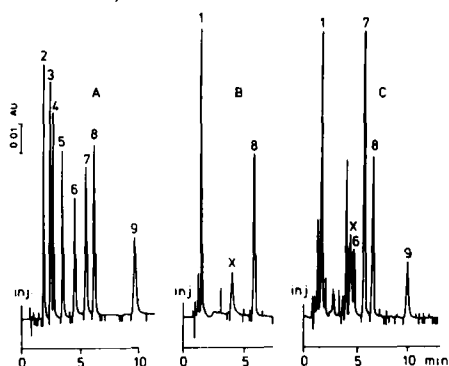


Fig. 11.8. Analysis of theophylline in the presence of caffeine and its metabolites¹⁹⁶
Precolumn Lichrosorb RP2 (45x2.0 mm ID), column Ultrasphere ODS 5 μ m (150x4.6 mm ID), mobile phase 0.01 M sodium acetate and 0.005 M tetrabutylammonium hydrogen sulfate in water (pH 4.75 with 0.1 M acetic acid or sodium hydroxide) to which 12.5% methanol is added, flow rate 1.5 ml/min, detection UV 274 nm. Peaks: 1, uric acid; 2, 3-methylxanthine; 3, 1-methylxanthine; 4, 1-methyluric acid; 5, 1,3-dimethyluric acid; 6, 1,7-dimethylxanthine; 7, theophylline; 8, β -hydroxyethyltheophylline (internal standard); 9, caffeine; x, unknown. Chromatogram A: standard mixture; chromatogram B: blank plasma; chromatogram C: plasma from patient taking theophylline and drinking coffee.

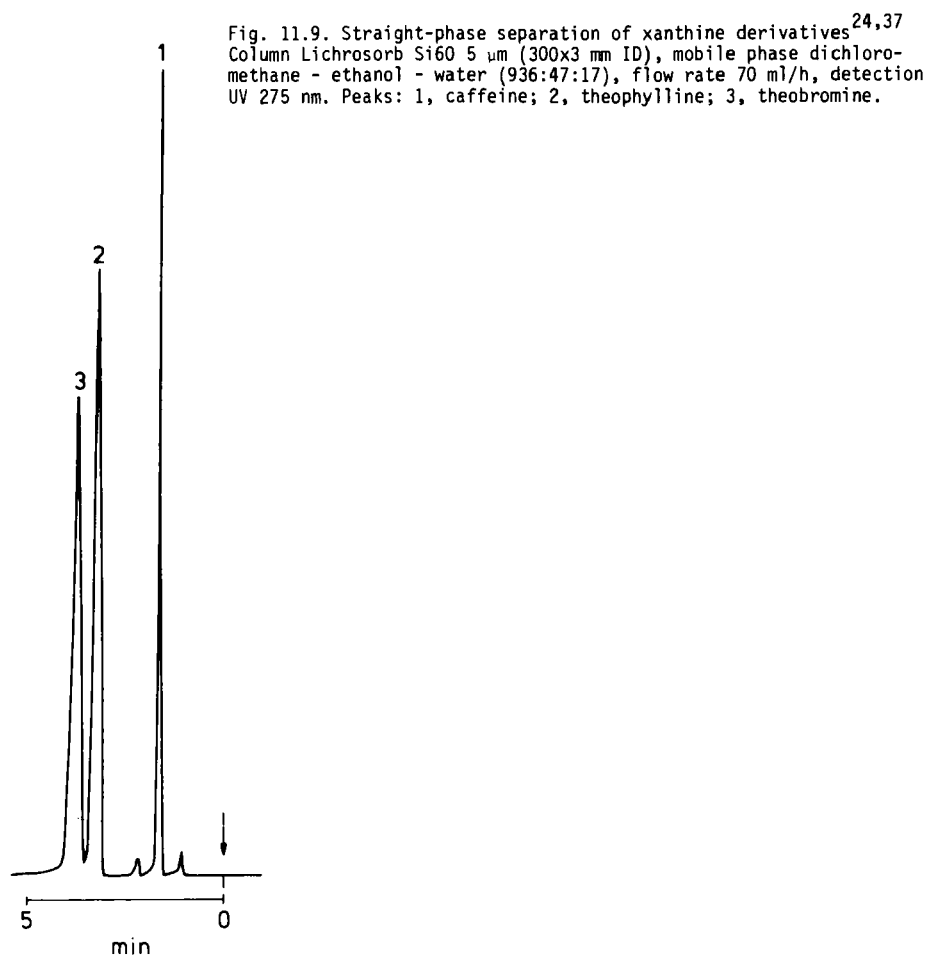


TABLE 11.5

XANTHINE ALKALOIDS IN THE CONTEXT OF HPLC ANALYSIS OF DRUGS OF ABUSE (CHAPTER 7)

Alkaloids*	Ref	Ref in Chapter 7	Alkaloids	Ref	Ref in Chapter 7
Caf	5	2	Caf	149	79
Caf	9	4	Caf	151	82
Caf	17	15	Caf	163	91(Table 7.11)
Caf	21	18(Fig.7.2)	Caf	164	93
Caf	22	21	Caf	167	97
Caf	23	22(Fig.7.16, Table 7.8)	Caf	182	106
Caf	45	30	Caf	184	108
Caf,Tp	60	38	Caf	199	113
Caf	73	44	Caf,Tp	201	118
Caf	114	55	Caf	203	120
Caf,Tb,Tp	115	56	Caf,Tp	204	121(Table 7.6)
Caf	143	73			

* Abbreviations used in Tables 11.5 - 11.9

Caf	Caffeine
Tb	Theobromine
Tp	Theophylline
1MeX	1-Methylxanthine
3MeX	3-Methylxanthine
7MeX	7-Methylxanthine
hypox	Hypoxanthine
paraX	Paraxanthine(1,7-dimethylxanthine)
X	Xanthine
dyp	Dyphylline
prox	Proxiphylline(β -hydroxypropyltheophylline)
OHETp	β -Hydroxyethyltheophylline
8ClTp	8-Chlorotheophylline
UA	Uric acid
1MeUA	1-Methyluric acid
3MeUA	3-Methyluric acid
7MeUA	7-Methyluric acid
1,3diMeUA	1,3-Dimethyluric acid
1,7diMeUA	1,7-Dimethyluric acid
3,7diMeUA	3,7-Dimethyluric acid
1,3,7triMeUA	1,3,7-Trimethyluric acid
A	Atropine
Acsal	Acetyl salicylic acid
Ampy	Aminopyrine
Antp	Antipyrine
Benzac	Benzoic acid
Eph	Ephedrine
Par	Paracetamol(acetaminophen)
Ph	Phenacetin
Phbarb	Phenobarbital
Sal	Salicylic acid
Salam	Salicylamide
Scop	Scopolamine

TABLE 11.6

HPLC ANALYSIS OF VARIOUS COMPOUNDS INCLUDING XANTHINE DERIVATIVES

ALKALOIDS *	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
Caf,Tb,Tp,strychnine, brucine		Separation by means of dy- namic coating HPLC	Corasil I or II, coated with 1.1% Poly G-300	1000x1	Heptane-EtOH(20:1),(10:1) sat. with stationary phase	4,7
Caf,various others		Detection with conductan- ce detector	Silica gel 10 μ m		CHCl ₃ -MeOH-hexane(7:3:10)	94
Caf,Tb,Tp,hypoX,X	Uracils,barbiturates	Retention in RP gradient elution LC	Lichrosorb Sil100, 10 μ m, C18 coated	300x4.2	MeOH-H ₂ O in various ratios	118
Caf,codeine,brucine, colchicine,aconitine, narceine,cinchonidine	Santonine	Effect solvent composition on retention	Lichrosorb RP2 10 μ m	120x3.5	MeOH-H ₂ O(1:4),(2:3),(3:2),(4:1) MeOH	121
Caf,tropane alkaloids, codeine,papaverine,qui- nidine,ephedrine		Retention behaviour basic drugs in ion-pair HPLC	μ Bondapak C18, μ Bondapak Phenyl, μ Bondapak CN, μ Bondagel, Chromegabond C8 or Chromegabond C ₆ H ₁₁	300x4	0.005M Heptanesulfonic acid in H ₂ O-MeOH-AcOH(50:49:1)	150
Caf,Tp	Par,Acsal,various hypnotics	Toxicological drug screen	PE/HS-5 C8	125x4.6	Gradient of 20-60% ACN in 0.05 M phosphate buffer(pH 4.4)	194
Caf,Tp,various alka- loids	Various drugs	Separation basic drugs with non-aqueous ionic solvents	Spherisorb S5W sil- ica	250x4.9	MeOH-hexane(85:15) containing 0.02% HClO ₄	201

TABLE 11.7

HPLC ANALYSIS XANTHINE DERIVATIVES IN PHARMACEUTICAL PREPARATIONS

ALKALOIDS *	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
Caf	Acsal,Salam,Ph,Par	Analysis analgetic tablets	Zipax anion-ex- changer	1000x2.1	0.005M NH ₄ NO ₃ in pH 9.2 buffer	1
Caf	Acsal,Salam,Ph,Par	Analysis analgetic tablets	LFS pellicular an- ion-exchange resin	3000x1	1.0M Tris buffer (pH 9.0)	2

*For abbreviations see footnote Table 11.5

Caf,Tb,Tp,1MeX,3MeX, 7MeX,X,hypoX,UA	Acsal,Ph	Separation by ligand-ex- change LC	Chelex 100, 200- 400 mesh, loaded with Cu ²⁺	500x10	1M NH ₄ OH, 3M NH ₄ OH	3
Caf,Tb,Tp,hypoX	Acsal,Salam,Ph,Par, Sal,aminophenols,phe- nol,acetanilides, ethylbenzoates,amino- benzoic acid methylester	Separation analgesics	Q-150S-NH ₄ cation- -exchanger AG 1-X8-C1 PVP resin	1500x2 1000x2	25% EtOH 25% EtOH IsoprOH	6
Caf,Tb,Tp,hypoX,X,UA	Acsal,Salam,nicoti- nic acid,trigonellin	Separation analgesics	Aminex 50W-X4	440x6	25% EtOH	10,15,38
Caf	Ph,propyphenazone	Analysis analgetic tablets	Merckosorb Si60 20 μm	200x2	CHCl ₃	11
Caf,homatropine,oxy- codone	Acsal,Ph,hexobarbi- tal	Analysis analgesics	Zipax WAX 30 μm	1000x2.1	1.5M Na ₂ SO ₄ ,0.005M HNO ₃ in H ₂ O	12
Caf	Acsal,Ph,Par,Benzac, butalbital,p-chloro- acetanilide	Analysis analgesics	Zipax SAX μBondapak C18	1220x2.3 300x4	0.01M Na-borate,0.01M NH ₄ NO ₃ in H ₂ O ACN-0.01% aq. (NH ₄) ₂ CO ₃	13
Caf	Acsal,Salam,Ph,Par	Analysis analgesics	Pore glass CPG-10- 240A,200-400 mesh Corasil II	600x2.1 600x2.1	CHCl ₃ -AcOH(92:8) CHCl ₃ -AcOH(92:8) CHCl ₃ -CH ₂ Cl ₂ -AcOH(42:50:8)	25
Caf	Ph,Ampy	Analysis analgesics	Zipax SCX	1000x2.1	A. 0.15M Na ₂ SO ₄ ,0.05M NaOH in H ₂ O B. MeOH 2%/min linear gradient 100% A to A+B(91:9)	29
Caf,Tp,papaverine	Ampy,nicotinamide, Phbarb	Analysis pharmaceutical preparations	Spherosil 5 μm	150x4.8	(Isopr) ₂ 0-MeOH-50% aq. ethyl- amine(93:6.86:0.14) EtOAc-MeOH-50% aq. ethylamine (97:2.94:0.06)	31
Caf	Antp,Ph,acetanilide	Analysis analgesics	ODS Sil-X-II	500x2.6	MeOH-H ₂ O(85:15),(1:4)	36
Caf,Tb,Tp,X,A,scop,er- gotamine,ergotamine	Butalbital	Separation on silver im- pregnated silica	Lichrosorb Si60 5 μm not 0.45% AgCl imp. given Lichrosorb Si100 10 μm 1.09% AgCl imp.		CHCl ₃ -n-hexane-MeOH(6:4:0.5) CHCl ₃ -DEA(99.99:0.01) A. CHCl ₃ -n-hexane(1:1) B. CHCl ₃ -MeOH-DEA(90:10:0.5) gradient 16-92% B in A	43
Caf,noscapine,dextro- morphane	Various compounds	Separation antitussives, expectorants and antihis- tamins	Porous styrene- divinylbenzene co-polymer	no de- tails available	MeOH-NH ₄ OH	52,58
Caf,Tb,Tp,various alkaloids	Various drugs	Separation various drugs	Partisil 10	250x4.6	CH ₂ Cl ₂ -MeOH(1:3)with 1% 29% NH ₄ OH	61

Caf	Acsal, Sal, Salam, Ph, Par	Analysis analgesics	Amberlite XAD-7	300x2.8	Et ₂ O-hexane in various ratios CHCl ₃ -hexane in various ratios EtOH-hexane in various ratios	70
Caf	Salam, Ph, fluoren	Analysis analgesics	Silica gel Si60	240x10	Hexane-dioxane-HCOOH(45:40:2)	85
Caf, tropane and ergot alkaloids	Phbarb, butalbital, barbital, pizotifene	Separation by ion-pair chromatography	Lichrosorb Si100 loaded with 0.06M picric acid(pH 6)	150x3	CHCl ₃ sat. with 0.06M picric acid(pH 6)	88
Caf, X	Acsal, Sal, Salam, Par, Ph, p-aminobenzoic-, nicotinic-, cinnamic-acid, trigonellin	Separation analgesics with cation-exchange resins	Aminex 50W-X4-NH ₄ ⁺ or TSK-LS110	200x6.3 or 250x4	25% EtOH in 0.1M buffer of various pH	90
Caf, Tb, Tp, 8ClTp, amino-phylline, A, Eph	Phenazone	Analysis analgesics	Hypersil ODS 5 μm	100x5	MeOH-aq. KH ₂ PO ₄ buffer(pH 6.0) (1:4)	92
Caf	Acsal, Par, Ph, mepro-bamate, methocarbamol, carisoprodol, chlorzoxazone	Separation muscle-relaxant-analgesic mixtures	μBondapak CN	300x4	1. THF-benzene(4:1) 2. THF-Bu ₂ O(65:35) 3. THF-CCl ₄ (1:1) CH ₂ Cl ₂ -CCl ₄ (95:5) CHCl ₃ -hexane(3:1) CHCl ₃ -Bu ₂ O(3:2) CHCl ₃ -CCl ₄ (3:2) eluent 1, 2 and 3	93
Caf, noscapine	Acsal, Sal, Par, ethoxybenzamide, various neuroleptics	Separation on porous polymer resins	DVB-MCL-0 and Hitachi gel 3011, 3011-0 and 3030	500x5	MeOH-NH ₄ OH(99:1) ACN-NH ₄ OH(99:1)	117
Caf, tropane and opium alkaloids, quinine, emetine, cephaeline, Eph, strychnine		Identification of pharmaceuticals(Fig.7.14)	Partisil PXS 5/25	250x4.6	Et ₂ O sat. with 50-100% H ₂ O + 0.05%-0.8% DEA	119
Caf, opium alkaloids		Analysis opium alkaloids	Partisil 7 μm	250x4.5	MeOH-2M NH ₄ OH-1M NH ₄ NO ₃ (30:2:1)	124
Caf	Ampy, Phbarb, nicotinamide	Separation	Spherosil XOA 600	50x4	Isooctane-(isopr) ₂ O-MeOH-H ₂ O (35:50:15:0.2:0.78)	130
Caf	Acsal, Sal, Ph, Par	Analysis analgesics	Partisil PXS 10/25 ODS2	250x4.6	ACN-H ₂ O-ACN(25:70:5)	145
Caf, codeine	Acsal, Salam, Ph, Par	Analysis analgesics	μBondapak C18	300x4	0.01M KH ₂ PO ₄ in H ₂ O with 19% MeOH pH 4.85 or adjusted 2.3	153
Caf, Tp	S-carboxymethyl-L-cysteine	Analysis in tablets and suppositories	Micropak M-CH 10	300x4	MeOH-H ₂ O(3:7) containing 0.005 M tetrabutylammonium(pH 7.5)	162

Tp,Eph	Phbarb	Analysis in tablets	Partisil ODS II ITP	250x4	MeOH-0.007M KH_2PO_4 (pH 2.3) (37:63)	172
Tp,Eph	Phbarb	Analysis in tablets	μ Bondapak C18	300x4	ACN-0.01M phosphate buffer (pH 7.8) (24:76)	173
Caf	Na-benzoate	Analysis in pharmaceuticals	Nucleosil C18	not avail-	MeOH-phosphate buffer	189
Tp,dioxyfylline		Analysis in tablets and syrups	Lichrosorb RP18 10 μ m	10 μ m	ACN- H_2O (3:7)	190
Tp,Eph	Hydroxyzine	Analysis in tablets	μ Bondapak C18	300x4.1	ACN-0.1% aq. $(\text{NH}_4)\text{CO}_3$ (pH 7.0) (1:1)	200
Caf	Saccharin,benzoic acid	Optimization separation by non-linear programming	Lichrosorb RP8 10 μ m	250x4.6	ACN-MeOH- H_2O in various ratios	202

TABLE 11.8

HPLC ANALYSIS OF XANTHINE DERIVATIVES IN BIOLOGICAL FLUIDS

ALKALOIDS*	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
Caf,Tb,Tp,1MeX,7MeX	Oxazepam	Assay of Tp in biological fluids	Durapak OPN	2000x4.2	Hexane-isoprOH gradient (86:14) to (78:22)	14
Caf,Tb,Tp,1MeX,3MeX,7MeX,hypoX,paraX,X,UA,1MeUA,3MeUA,1,3diMeUA		Determination Tp and its metabolites in urine and serum (Table 11.1)	Aminex A-5 cation-exchanger	665x1.8	0.45M $\text{NH}_4\text{H}_2\text{PO}_4$ (pH 3.65)	16
Caf,Tb,Tp,3MeX,hypoX,X,OHETp,Dyp,8ClTp,Tp-7-acetic acid,UA,1MeUA,1,3diMeUA		Assay of Tp in serum	Aminex A-5 cation-exchanger	850x1.6	0.45M $\text{NH}_4\text{H}_2\text{PO}_4$ (pH 3.65)	18
Caf,Tb,Tp,3MeX,1,3diMeUA		Assay of Tp in plasma	Micropak Si10 10 μ m	500x3	CHCl_3 -isoprOH-AcOH (84:15:1)	19
Caf,Tp	Morphine,codeine,cocaine	Determination in urine	BOP(no further details)		Heptane-prOH (9:1)	20
Caf,Tb,Tp,1MeX,3MeX,X,hypoX,paraX,dyp,prox,8BrTp,8ClTp,8NO $_2$ Tp,8ClX,UA,1MeUA,1,3diMeUA	Phbarb	Assay of Tp in biological fluids	Zipax SCX	1000x2.1	0.66% aq. AcOH	26
Caf,Tp,1MeX,3MeX,7MeX,hypoX,X,UA,1MeUA		Assay of Tp in plasma	μ Bondapak C18	300x4	ACN-0.01M NaOAc (pH 4.0) (1:9)	27

*For abbreviations see footnote Table 11.5

Tp, OHETp		Assay of Tp in serum	μ Bondapak C18	300x4	ACN-0.01M NaOAc (pH 4.0) (7:93)	28
Caf, Tp	Phbarb	Assay of Tp in plasma	Partisil 10	450x2	CH ₂ Cl ₂ -MeOH-28% NH ₄ OH (92:7:1)	30
Caf, Tb, Tp, 8ClTp, dyp, prox		Assay of Tp in plasma	Zorbax Sil 6-8 μ m	250x2.1	H ₂ O sat. CHCl ₃ -heptane-EtOH-AcOH (600:400:0.8:64)	33
Caf, Tb, Tp, 3MeX, hypoX, X, OHETp, 8ClTp, 1, 3diMeUA		Assay Tp in serum	ODS Sil-X-I 13 μ m	250x2.6	ACN-H ₂ O-1% aq. AcOH (1:48:1) (pH 4.5)	35
Caf, Tb, Tp, OHETp, 8ClTp		Assay Tp in blood and saliva	μ Bondapak C18	300x4	ACN-0.01M NaOAc (pH 4) (7:93), (4:96)	39, 109
Caf, Tb, Tp, prox, dyp	Phbarb	Assay Tp and dyp in serum	Partisil 10	250x4.6	CHCl ₃ -n-heptane-MeOH (39:56:5) followed by (37:54:5)	44
Caf, Tb, Tp, 3MeX, X	Purine nucleotides	Analysis in biological extracts	μ Bondapak C18	300x4	MeOH-0.05M NH ₄ H ₂ PO ₄ (pH 6.0) (1:3)	46
Caf, Tb, Tp, 3MeX, 1MeUA, 1, 3diMeUA		Assay Tp in plasma	μ Porasil	300x4	n-Hexane-sec-BuOH-MeOH-H ₂ O (69:25:5:1)	48
Caf, Tb, Tp		Assay Tp in plasma and saliva	Partisil SCX	250x4.6	0.66% aq. AcOH	49
Caf, Tb, Tp, paraX		Identification caf metabolites in plasma (Table 11.4)	Lichrosorb Si60 5 μ m	250x3	CHCl ₃ -isoprOH-AcOH (92:7:1) + up to 40% n-hexane CH ₂ Cl ₂ -(0.2% NH ₄ formate and 15 μ l 80% HCOOH in 100 ml MeOH) (98:2)	51
Tp	Prednisolone	Assay of Tp in plasma, comparison with GLC (Table 11.4)	Lichrosorb Si60 5 μ m	250x3.2	CHCl ₃ -isoprOH-AcOH (94:5:1) CH ₂ Cl ₂ -(0.2% NH ₄ formate and 0.02% HCOOH in MeOH) (98:2)	53
Caf, Tb, Tp, 1MeX, 3MeX, X, hypoX, prox, UA, 1MeUA, 3MeUA, 1, 3diMeUA		Assay Tp in serum (Table 11.3)	μ Bondapak C18	300x4	ACN-0.02M NaOAc (pH 4.0) (1:9)	54
Tp, dyp, 8ClTp		Assay Tp in plasma	Partisil 10 ODS	250x4.6	ACN-0.01M KH ₂ PO ₄ (9:1)	55
Caf, Tb, Tp, 3MeX, OHETp, 8ClTp, 1MeUA, 1, 3diMeUA	Phbarb	Assay Tp in plasma, serum and saliva (Fig. 11.1)	μ Bondapak C18	300x4	ACN-0.01M NaOAc (pH 4.0) (7:93) or (5:95)	56
Tb, Tp, dyp, prox		Assay dyp in serum	μ Bondapak C18	300x4	ACN-0.01M NaOAc (pH 4.0) (7:93)	57
Caf, Tb, Tp, 8ClTp, dyp		Assay Tp in whole blood and serum	Spherisorb ODS 10 μ m	250x2.6	MeOH-0.01M NaOAc (pH 4) (1:3)	59
HypoX, X, allupurinol, oxipurinol		Determination in plasma and urine	Micropak Si 10 μ m	500x2	A. Hexane-isoprOH (10:1) B. Hexane-isoprOH-conc. NH ₄ OH (5:50:4:1), 67% B in A	62

Tb, Tp, 1MeX, 3MeX, hypoX, X, dyp, UA, 1MeUA, 1, 3diMeUA		Analysis Tp and metabolites in serum, saliva and urine (Fig.11.3)	μBondapak C18	300x3.9	MeOH-0.05M KH_2PO_4 (pH 4.7) (12:88)	63
Caf, Tb, Tp, 8ClTp		Assay Tp in serum	μBondapak C18	300x4	MeOH-1% propionic acid in H_2O (pH 5.0 with NaOH) (1:4)	64
Caf, Tb, Tp		Assay Tp in serum	μBondapak C18	300x4	MeOH-0.01M NaH_2PO_4 (1:4)	65
Caf, Tb, Tp, 1MeX, 3MeX, 7MeX, paraX	Prednisolone	Assay caf metabolites in plasma (Table 11.4)	Lichrosorb Si60 5 μm	250x3	CH_2Cl_2 -(0.02g NH_4 formate and 0.617g 97% HCOOH in 100 ml MeOH) (978:22)	67
Caf, Tb, Tp, 3MeX, prox, dyp, 8ClTp		Assay xanthines in serum (Fig.11.4)	μBondapak C18	300x3.9	MeOH-0.02M KCl (pH 2 with HCl) (3:7)	68
Tp, prox		Comparison method ref. 26 with EMIT	Zipax SCX	1000x2.1	0.66% aq. AcOH	69
Caf, Tp		Assay Tp in biological fluids	Spherisorb ODS 5 μm	150x4.6	MeOH- H_2O (2:3)	71
Caf, Tb, Tp, OHETp		Comparison Tp assays (HPLC and EMIT)	Hypersil ODS 5 μm	100x5	ACN-0.01M NaOAc (pH 4.0) (7:93)	72
Tp, 8ClTp		Assay Tp in saliva	ODS-I	250x2.6	MeOH-0.2% H_3PO_4 (pH 4 with NaOH) (1:4)	74
Caf, Tb, Tp, 1MeX, 3MeX, X, UA, 1MeUA, 3MeUA, 1, 3diMeUA	Furosemide, phbarb, sal, sulfathiazole, chlorothiazide	Assay of Tp in serum	Partisil 5 μm	250x2.1	Hexane-isoprOH- H_2O (80:19:1)	75
Caf, Tb, Tp, 3MeX, dyp, 8ClTp, 1MeUA, 1, 3diMeUA		Assay of Tp in plasma	ODS HC Sil-X-1 μBondapak C18	not given	ACN- H_2O (1:9) not given ACN- H_2O (6:94)	76
Tp	Cephalosporin antibiotics	Interference of Tp analysis	Partisil 10 ODS	250x4.6	ACN-0.1M NaOAc (pH 4.0) (1:9)	77
Caf, Tb, Tp, OHETp		Assay of Tp in serum	μBondapak C18	300x4	ACN-0.026M NaOAc (pH 4.0) (1:9)	79
Caf, Tb, Tp, 1MeX, 3MeX, 7MeX, paraX, X, dyp, 8ClTp, UA, 1MeUA, 3MeUA, 7MeUA, 1, 3diMeUA	Creatinine	Amperometric detection of methylxanthines (Fig.11.2)	μBondapak C18	300x4	ACN-(0.1M Na_2HPO_4 , 0.1M NaH_2PO_4) (2:38) ACN-0.1M NaH_2PO_4 (21:400)	80
Tp, OHETp	Cephalosporins	Interference of Tp analysis	μBondapak C18	300x4	ACN-0.01M NaOAc (pH 4.0) (7:93)	81
Tp, OHETp	Acetazolamide	Interference of Tp analysis	μBondapak C18	300x4	ACN-0.01M NaOAc (pH 4.0) (7:93)	82
Tp		Assay of Tp in serum	μBondapak C18	300x4	ACN-0.01M NaOAc (pH 4.0) (8:92)	84
Tb, Tp, hypoX, dyp, 8ClTp		Assay of Tp in serum	Partisil 10 ODS	250x4.6	MeOH-0.025M KH_2PO_4 (pH 2.5) (35:65)	91

Tp,paraX,OHETp		Assay of Tp in serum	μBondapak C18	300x4	ACN-0.01M NaOAc(pH 4.0)(1:9)	95
Caf,Tb,Tp,paraX	Chloramphenicol	Assay of Tp in serum and saliva	Lichrosorb Si60 7 μm	100x2.8	CH ₂ Cl ₂ -(0.02% NH ₄ formate, 0.017% HCOOH in MeOH)(99:1)	96
Tp		Comparison Tp assays (HPLC and EMIT)	Aminex A5 cation-exchange resin	850x1.6	0.45M NH ₄ H ₂ PO ₄ (pH 3.65)	97
Caf,Tb,Tp,1MeX,3MeX,7MeX,hypoX,paraX,X,OHETp,1MeUA,3MeUA,1,3MeUA	Par,heptabarbital,phbarb	Comparison Tp assays (HPLC and GLC)	Hypersil ODS 5 μm	100x5	ACN-0.02M NaOAc(pH 4.0)(8:92)	98
Caf,Tp,8ClTp		Assay of Tp in plasma	μBondapak C18	300x4	MeOH-0.05M NH ₄ H ₂ PO ₄ (pH 5.2)(74:26)	99
Tp		Comparison Tp assays (HPLC and EMIT)	Micropak MCH-10	not given	CHCl ₃ -isoprOH-AcOH(4:5:1)	100
Tp,OHETp	Acetazolamide	Interference of Tp analysis	μBondapak C18	300x4	ACN-0.003M NaOAc(pH 4.5)(8:92)	101
Tp,dyp,OHETp	Sulfadiazine	Interference of dyp analysis	μBondapak C18	300x4	ACN-0.01M NaOAc(pH 4.0)(7:93)	102
Caf,Tb,Tp,dyp,8ClTp		Assay of Tp in plasma	μBondapak C18	300x4	ACN-1M KH ₂ PO ₄ (pH 4.0)-H ₂ O(50:5:895)	103
Caf,Tb,Tp,1MeX,3MeX,7MeX,OHETp,1MeUA,1,3diMeUA		Assay of Tp in serum	μBondapak C18	300x4	MeOH-0.02M NaOAc(pH 3.5)(15:85)	104
Caf,Tb,Tp,3MeX,dyp,1MeUA,1,3diMeUA		Assay of Tp in plasma	PSX 10/25 ODS	not given	95% EtOH-H ₂ O(1:4)	105
Caf,Tb,Tp,paraX,OHETp		Interference Tp assay by caf metabolite (paraX)	μBondapak C18	300x4	MeOH-THF-0.01M NaOAc(pH 5.0)(95:4:1)	106
Caf,Tb,Tp,1MeX,3MeX,7MeX,paraX,1MeUA,3MeUA,7MeUA,1,3diMeUA,1,7diMeUA,3,7diMeUA,1,3,7triMeUA		Caf metabolism in newborns	μBondapak C18 (RP-10 in ref 133)	300x4	ACN-0.5% AcOH, concave gradient from (15:985) to (75:925)	107,133
Tp,1MeX,3MeX,8ClTp,1MeUA,1,3diMeUA		Tp metabolism	C-18 stationary phase(no details) 5 μm	150x4	ACN-0.01M NaOAc(pH 4.0)(12:88)	108
Caf,Tb,Tp,dyp,prox		Assay of Tp in serum	Partisil 10 ODS	250x4.6	MeOH-0.049M phosphate buffer (pH 2.3)(2:3)	109
Caf,Tb,Tp		Comparison Tp assays in serum,saliva and spinal fluids with HPLC or EMIT	Lichrosorb Si60 5 μm	250x3.2	CHCl ₃ -isoprOH-AcOH(96:2:2)	110
Caf,Tb,Tp,OHETp,8ClTp		Assay of Tp in serum,plasma and saliva	μBondapak C18	300x4	ACN-0.01M NaOAc(pH 4.0)(7:93) or (4:96)	111

Caf,Tb,Tp,3MeX,paraX, OHEtTp,prox,8ClTp, 1MeUA,1,3diMeUA		Assay of Tp in serum, com- parison with GLC (Table 11.3)	Hypersil ODS 5 μ m	100x5	ACN-0.02M NaOAc(pH 4.0) (8:92)	112
Caf,1-propylTp		Caf determination in plasma or tissues	ODS-Sil-X-1 10 μ m	250x2.6	ACN-H ₂ O(3:1)	113
Caf,Tb,Tp,8ClTp		Electrochemical detection Tp	Lichrosorb C8 10 μ m	250x3.2	EtOH-NaOAc buffer(pH 4.0) (8:92)	116
Caf,Tp,hypoX		Analysis on cation-exchange resins	Aminex 50W-X4	150x4.6	0.05M Na ₂ HPO ₄ (pH 7.5)	120
Caf,Tb,Tp,1MeX,paraX, OHEtTp,dyp,1MeUA, 1,3diMeUA	Ampicillin	Assay of Tp	Partisil PXS 5/25 ODS	250x5	MeOH-THF-0.01M NaOAc(pH 5.0) (92:7:1)	122
			RCM with Radial Pak A (C18)		MeOH-THF-0.01M NaOAc(pH 5.0) (928:60:12)	122
Dyp,OHEtTp		Urinary excretion of dyp	μ Bondapak C18	300x3.9	ACN-0.01M NaOAc(65:935)	123
Caf,Tb,Tp,dyp,OHEtTp		Assay of dyp in plasma, urine and saliva	μ Bondapak C18	300x4	ACN-H ₂ O(9:91)	125
Caf,Tb,Tp,dyp,prox		Assay of Tp in plasma, urine and saliva	MPLC RP18	100x4.6	MeOH-H ₂ O(1:4).	126
Caf,Tb,Tp,1MeX,3MeX, dyp,8ClTp,prox,UA		Comparison Tp assays(HPLC and EMIT)	Micropak SI 5 5 μ m	250x2	CHCl ₃ -heptane-AcOH-EtOH (300:200:0.4:32)	127
Caf,Tb,Tp,1MeX,3MeX,X, hypoX,8ClTp,UA,1MeUA, 3MeUA		Assay of Tp in serum and possible interferences (Fig.11.5)	Micropak MCH-10	not given	IsoprOH-0.1M KH ₂ PO ₄ (pH 3.8) (4:96)	128
Caf,Tb,Tp,OHEtTp		Assay of Tp in body fluids	Hypersil ODS 5 μ m	100x5	ACN-NaOAc buffer(pH 4.0) (8:92)	129
Caf,Tb,Tp,8ClTp		Simultaneous assay caf, Tb and Tp in plasma, compari- son with EMIT	S5 ODS	150x4.6	ACN-0.01M NaOAc(pH 4)(12:88)	136
Caf,Tb,Tp,1MeX,3MeX, 7MeX,hypoX,paraX,X,dyp, prox,UA,1MeUA,7MeUA, 1,3diMeUA		Assay of caf in plasma (Table 11.3)	Spherisorb ODS 10 μ m	250x4.6	ACN-0.01M NaOAc(pH 4.0)(15:85), (18:82)	138
Tp	Sulfamethoxazole	Interference Tp assay	Lichrosorb RP8	250x4.6	ACN-0.005M AcOH(1:3)	139
Caf,Tb,Tp,8ClTp	Sulfamethoxazole,am- picillin,par,Acsal, sal,various antibiotics	Interference Tp assay	Lichrosorb RP8	250x4.6	MeOH-0.02M NaOAc(pH 5.5)(1:4)	140,191
Caf,Tb,Tp,1MeX,paraX, 1MeUA,1,3diMeUA	Carbamazepine	Assay of caf in serum	Partisil 5 5 μ m	100x4.6	THF-CH ₂ Cl ₂ (1:4)	144

Caf, Tp, 1MeX, 3MeX, paraX, OHETp, 1MeUA, 1, 3diMeUA		Assay of Tp and metabolites in urine	Ultrasphere ODS 5 μ m	250x4.6	A. 0.01M NaOAc, 0.005M tetra-butylammonium sulfate in H ₂ O B. idem in 50% MeOH gradient from 4.5-23% MeOH	146
Caf, Tb, Tp, dyp, OHETp		Assay Tp and dyp in plasma	μ Bondapak C18	300x4	ACN-0.01M NaOAc(6:94)	147
Caf, Tb, Tp, bamifylline, dyp, prox, lomifylline, pentifylline, pentoxifylline		Separation and analysis in plasma	Spherisorb C6 5 μ m, Spherisorb CN 5 μ m or Spherisorb ODS 5 μ m Zorbax Sil 7 μ m	200x4.6 200x4.6	ACN-0.01M phosphate buffer (pH 2.7)(1:4), (28:72) Hexane-CHCl ₃ -isoproH-AcOH (50:43:5:2) ³	152
Tp, 30HpropylTp		Comparison Tp-assays (HPLC and RIA)	RP-8	125x4.6	ACN-0.04M NaOAc(pH 4.0)(5:95)	155
Caf, Tb, Tp, 3MeX, paraX, 1MeUA, 3MeUA		Assay Tp in serum and saliva	Spherisorb ODS 10 μ m	250x3	Heptane-CHCl ₃ -EtOH-H ₂ O-AcOH (400:600:32:1.5:0.8) ²	156
Caf, Tb, Tp, 1MeX, 3MeX, X, Par, ethosuximide hypoX, OHETp, 8ClTp, UA, 1MeUA, 3MeUA		Simultaneous analysis in plasma and comparison with EMIT	μ Bondapak C18	300x3.9	ACN-H ₂ O-TrEA or N-ethylmorpholine-2-AcOH(1:11:0.008:0.006)	157
Caf, Tb, Tp, 1MeX, 3MeX, 7MeX, hypoX, X, paraX, prox, UA, 1MeUA, 7MeUA, 1, 3diMeUA		Interference benzoic acid with method ref.138 (Table 11.3)	Ultrasphere ODS 5 μ m	150x4.6	ACN-0.01M acetate buffer (pH 6.5)(9:91)	158
Tp, OHETp		Artefacts with acetonitrile formed by deproteination of serum	Radial-Pak C18	100x8	ACN-0.01M NaOAc(1:9)	159
Caf, Tb, Tp, 3MeX, 8ClTp	Par, salicylate, mephensine, acetophenetidine	Assay of Tp in serum	Spherisorb ODS 10 μ m	250x3	ACN-MeOH-H ₂ O-10ml/l aq.AcOH (16:180:788:16)	160
Caf, Tb, Tp	Ampicillin	Assay of Tp in serum and saliva	μ Bondapak C18	300x4	ACN-H ₂ O(8:92)	161
Caf, Tb, Tp, paraX, 3-isobutyl-1MeX		Assay of Tp in plasma, comparison with RIA	RSil, silica gel 5 μ m	100x2.8	CHCl ₃ -dioxane-HCOOH(995:45:0.1)	165
Caf, Tb, Tp, paraX, OHETp		Determination caf and its metabolites in dogs plasma	μ Bondapak C18	300x3.9	ACN-MeOH-THF-0.005M NaOAc buffer(pH 5)(28:30:17:925)	167
Caf, Tb, Tp, 1MeX, 3MeX, OHETp, UA, 1, 3diMeUA		Assay of Tp in plasma	μ Porasil	300x3.9	Hexane-EtOH(76:24)	168
Tp		Assay of Tp in plasma	No details available			174
Tp	Procainamide	Interference in Tp assay	μ Bondapak C18	300x3.9	ACN-0.01M NaOAc buffer(pH 4.0)(7:93)	176

Tp, paraX		Interference paraX in Tp assay	Lichrosorb RP18 7 μ m	300x4	MeOH-FMA-0.05M KH_2PO_4 (22:11.5:66.6) (pH 5.8)	177
Caf, Tp, 8C1Tp		Analysis Tp in biological fluids	ODS-type	no details available	ACN-0.1M phosphate buffer (pH 5.3) (1:9)	178
Tp		Direct injection of plasma samples on column	TSK LS-410, 20-32 μ m, treated with human plasma	60x4	ACN-phosphate buffer (pH 7.4)	181
Caf, Tb, Tp, 3MeX, paraX, OHETp, dyp, 8C1Tp,	Par, acetazolamide, procainamide, various others	Fast analysis Tp in serum	C18 type 5 μ m	125x4.6	ACN-0.02M phosphate buffer (pH 3.6) (95:905)	183
Caf, Tb, Tp, 3MeX, OHETp, 8C1Tp, dyp	Par, procainamide, N-Ac-procainamide, N-propionylprocainamide, Sal, Acsal	Simultaneous assay of some drugs in serum	μ Bondapak C18	300x3.9	ACN-0.1M phosphate buffer (pH 4.0) (97.5:902.5)	187
Caf, Tb, Tp, 1MeX, 3MeX, 7MeX, paraX, X, OHETp, 1MeUA, 3MeUA, 7MeUA, 1,3-diMeUA, 3,7-diMeUA, 1,7-diMeUA, 1,3,7-triMeUA		Assay for simultaneous quantitation xanthines and uric acids in urine	Ultrasphere ODS 5 μ m	250x4.6	A. 0.01M NaOAc, 0.005M tetrabutylammonium in H_2O (pH 4.9) B. as A in 50% MeOH (pH 4.8) non-linear gradient elution	192
Caf, Tp	Par	Automated HPLC-analysis in serum	Fast LC-8 5 μ m	150x4.6	MeOH-0.0025M NaH_2PO_4 (14:86) with 0.065% TrEA (pH 6.6)	193
Caf, Tb, Tp, 3MeX, OHETp		Analysis caf in biological samples and coffee	ODS 5 μ m	250x4.6	ACN- H_3PO_4 - H_2O (260:1:1739)	195
Caf, Tb, Tp, 1MeX, 3MeX, paraX, OHETp, UA, 1MeUA, 1,3-diMeUA		Analysis Tp in plasma and saliva in the presence of caffeine and its metabolites	Ultrasphere ODS 5 μ m	150x4.6	MeOH-0.01M NaOAc, 0.005M tetrabutylammonium (pH 4.75) (1:7)	196
HypoX, X, UA		Specific detection with xanthine oxidase reactor	Nucleosil 5 C18	200x4	ACN-0.01M phosphate buffer (pH 5.5) (1:99)	197
9MeX, hypoX, X, UA, adenine, guanine, allopurinol		Analysis in biological fluids	Hypersil ODS 3 μ m	150x4.6	0.02M KH_2PO_4 (pH 3.65)	198
Caf, Tp, OHETp		Comparison HPLC columns	μ Bondapak C18 Hi-Chrom Reversible ODS-C18 5 μ m Partisil PXS 5/25 ODS Zorbax ODS 5 μ m	300x4.0 250x4.6 250x4.6 250x4.6	ACN- H_2O -AcOH (5:95:0.2)	208

TABLE 11.9

HPLC ANALYSIS XANTHINE DERIVATIVES IN FOOD AND BEVERAGES

ALKALOIDS*	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
Caf,Tb,Tp,1MeX,3MeX,7MeX, X,hypoX,UA	Determination caf in beverages	Chelex 100, 200-400 mesh, loaded with Cu ⁺⁺	500x10	1M NH ₄ OH 3M NH ₄ OH	3
Caf,Tb,Tp,X	Determination food additives	Zipax SAX	1000x2.1	0.01M Na-borate buffer(pH 9.2)	8
Caf,Tb,Tp,hypoX,X,UA,tri- gonellin,nicotinic acid	Analysis coffee	Aminex 50W-X4, NH ₄ ⁺	440x6	NH ₄ -formate buffer(pH 3.65) in 25% EtOH	10,15,38
Caf,Tb,Tp	Analysis of food and beverages (Fig.11.9)	Lichrosorb Si60 5 µm or Nucleosil 50 5 µm	300x3 300x3	CH ₂ Cl ₂ -EtOH-H ₂ O(936:47:17) organic phase ²	24,37
Caf	Analysis in food	ODS Si1-X-1	250x2.6	ACN-H ₂ O(2:98)	32
Caf,Tb,Tp,hypoX,trigone1- lin,nicotinic acid	Analysis in coffee	Zipax SCX	1000x2.1	aq. HNO ₃ (pH 1.56)	40
Caf	Determination caf, saccharin and sodium benzoate in beverages	µBondapak C18	300x4	5% aq. AcOH	41
Caf	Analysis coffee and tea	Zipax SCX	300x4	0.01M HNO ₃ (pH 2)	42
Caf,Tb,Tp	Analysis tea constituents	µBondapak C18	300x4	MeOH-0.1M citrate-phosphate buffer (pH 7.0)(1:4)	47
Caf,Tb	Analysis cocoa and chocolate	µBondapak C18	300x4	MeOH-H ₂ O-AcOH(20:79:1)	86,141,148
Caf,Tb,Tp,X,adenine	Analysis tea	Dowex AG-50W-X8 (H ⁺) cation-exchanger	600x9	25% EtOH	87
Caf,Tb	Analysis in cocoa beans	µBondapak C18	300x4	ACN-H ₂ O(15:85) containing 1%(NH ₄) ₂ HPO ₄	90
Caf,Tb,Tp,8ClTp	Analysis in pharmaceutical raw products (tea, cola nuts, etc.)	µBondapak C18	300x4.6	MeOH-H ₂ O(2:3)	131
Caf,Tp	Analysis in cocoa	no details available			134
Tb	Analysis in animal feeding stuffs	Partisil PXS 10/25	250x4.6	MeOH-4% aq. AcOH(1:4)	142
Caf,Tb,Tp,isobutylX	Influence brewing method on caf content coffee	Partisil PXS 10/25 ODS	250x4.6	MeOH-H ₂ O(1:4)	175
Caf	Analysis decaffeinated coffee	µBondapak C18	400x6	MeOH-H ₂ O(7:3)	180
Caf,Tb,Tp	Analysis animal diets	Radial-Pak C18	100x8	MeOH-H ₂ O-AcOH(25:74:1)	205
Caf,Tb,Tp	Analysis coffee,tea and cola nuts	Lichroprep Si60	240x10	CHCl ₃ -EtOH-25%NH ₄ OH(90:10:0.25)	206
Caf,Tb,Tp,3MeX,X,dyp, OHETp	Analysis cocoa content in milk- powder products	µBondapak C18	300x4	MeOH-H ₂ O-AcOH(19:80:1)	207

* For abbreviations see footnote Table 11.5

Chapter 12

DITERPENE ALKALOIDS

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12.1. HPLC SYSTEMS

Only a few data have been published on the HPLC of aconitine type of alkaloids (Table 12.1). Sheu et al.¹ analyzed aconitine in crude drugs on an octadecyl stationary phase using methanol - aqueous phosphate buffer (pH 7.52) (85:15) as mobile phase. The influence of the water - methanol ratio in the mobile phase on the retention of alkaloids, including aconitine, has been investigated on a reversed-phase packing³.

Hikino et al.⁴ developed two HPLC methods for the analysis of aconitine and related alkaloids in crude drugs. In the first one the alkaloids were separated on an octadecyl column using tetrahydrofuran - 0.05 M phosphate buffer (11:89) as mobile phase. Mobile phases containing methanol and acetonitrile gave broader peaks and less resolution of some of the alkaloids. In the pH range 2-5, little variation in k' was found for the alkaloids, but above pH 5 some alkaloids showed increased k' values. Best results were obtained at pH 2.7 (Fig. 12.1). A second method was developed to minimize the risk of interference of co-eluting compounds from the plant material. The same type of column as above was used in the reversed-phase ion-pair mode, and, as pairing-ion, 0.01 M hexanesulfonate was added to the mobile phase (tetrahydrofuran - 0.05 M phosphate buffer (pH 2.7)(15:85)).

A straight-phase HPLC method was developed by Gimet and Filloux². The alkaloids, including aconitine, were separated on silica gel using a mobile phase of water saturated diethyl ether, to which some diethylamine was added (see Chapter 7, Fig.7.14).

The detection of aconitine-type alkaloids has been performed at 254 nm, although the maximum UV absorption is at 235 nm.

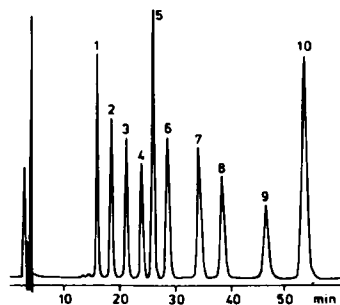
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TABLE 12.1

HPLC ANALYSIS ACONITINE AND RELATED ALKALOIDS

ALKALOIDS	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
Aconitine		Analysis crude drugs	Zorbax ODS	250x4.6	MeOH-aq. phosphate buffer(pH 7.42) (85:15)	1
Aconitine,opium-, tropane alkaloids, strychnine,quinine,caffeine,emetine,cephaeline	Sulfanilamide, phenytoine, phenobarbital	Identification in pharmaceuticals(Fig.7.14)	Partisil PXS 5/25	250x4.6	Et ₂ O sat. with 50-100% H ₂ O + 0.05-0.8% DEA	2
Aconitine,colchicine,caffeine,narceine,codeine,brucine,cinchonidine	Santonine	Effect solvent composition on retention	Lichrosorb RP2 10 μ m	120x3.5	MeOH-H ₂ O(1:4),(2:3),(3:2),(4:1) MeOH	3
Aconitine,mesaconitine,desoxyaconitine,hypaconitine,jesaconitines and the corresponding benzoylaconines		Analysis crude drugs(Fig. 12.1)	TSK Gel LS 410 ODS Sil 5 μ m	300x4	THF-0.05M phosphate buffer(pH 2.7) (11:89) 0.01M hexanesulfonate in THF-0.05M phosphate buffer(pH 2.7)(15:85)	4

Fig. 12.1. Separation of some aconite alkaloids⁴

Column TSK Gel LS410 ODS Sil 5 μ m (300x4 mm ID), mobile phase tetrahydrofuran - 0.05 M aqueous phosphate buffer (pH 2.7) (11:89), flow rate 0.9 ml/min, detection UV 254 nm. Peaks: 1, benzoylmesaconine; 2, benzoylaconine; 3, benzoylhypaconine; 4, benzoylmesaconine; 5, benzoylmesaconine; 6, mesaconitine; 7, hypaconitine; 8, aconitine; 9, desoxyaconitine; 10, jesaconitine.

Chapter 13

COLCHICINE AND RELATED ALKALOIDS

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13.1. HPLC SYSTEMS

Forni and Massarani¹ described the analysis of colchicine and colchicoside in plant material. They used a silanized silica gel as stationary phase and a gradient of acetonitrile and water as eluent. Assay of the alkaloids in plant material has been performed on a microparticulate octyl column with methanol - water as mobile phase². The best separation of a series of colchicine derivatives was obtained on an octadecyl column with acetonitrile - methanol - phosphate buffer as mobile phase (Fig.13.1)^{5,7,8}.

Optimum pH for the separation was 6, and the addition of methanol improved the peak shape. The method had to be modified for the analysis of colchiceinamide and its demethylated metabolites⁹. By using smaller particles for the stationary phase, the resolution could be improved. Peak shape was improved by adding triethylamine hydrochloride to the mobile phase. Acidifying the mobile phase to pH 2.2 resulted in resolution of colchicine and colchiceinamide.

Jarvie et al.³ used a microparticulate silica gel column for the determination of colchicine in plasma. Iorio et al.⁶ isolated and identified some impurities of colchicine by means of HPLC, TLC and MS. Alkaloid separation was performed on silica gel with a gradient system of chloroform - methanol.

Detection of colchicine and derivatives is best performed at its UV absorption maxima at 350 nm^{2,5,7} or 240 nm³.

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TABLE 13.1

HPLC ANALYSIS COLCHICINE AND DERIVATIVES

ALKALOIDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
Colchicine,colchicoside	Analysis colchicum seeds	Silanized Lichrosorb Si60 30 μ m	500x3	A. ACN B. ACN-H ₂ O(1:9) gradient ^a B to 30% A in B	1
Colchicine,colchicoside, 3-demethylcolchicine	Analysis of plant material	Lichrosorb RP8 10 μ m	250	MeOH-H ₂ O(1:2)	2
Colchicine	Determination in plasma	Hypersil 5 μ m	100x5	CH ₂ Cl ₂ -isoprOH-NH ₄ OH(ratio unspecified)	3
Colchicine,caffeine,narceine, codeine,brucine,cinchonidine, aconitine	Effect solvent composition on retention	Lichrosorb RP2 10 μ m	120x3.5	MeOH-H ₂ O(1:4),(2:3),(3:2),(4:1) MeOH	4
Colchicine,colchicine,demecol- cine,N-methyl-colchicineamide, colchicine,ethylisocolchicinate, ethylcolchicinate	Determination in microbial cultures	Lichrosorb RP18	100x4.1	ACN-MeOH-phosphate buffer(pH 6) (16:5:79)	5
Colchicine,17-hydroxycolchici- ne,colchicine, β -lumicolchi- cine,N-formyl-desacetylcolchicine	Identification impurities in colchicine	Lichrosorb Si100 10 μ m	250x4.5	A. CHCl ₃ sat. with H ₂ O B. CHCl ₃ -MeOH(95:5) sat. with H ₂ O gradient 10% B to 100% B in A	6
Colchicine,demecolcine,N-des- acetylcolchicine,3-demethyl- colchicine,2-demethylcolchi- cine,1-demethylcolchicine	Analysis in biological mate- rial(Fig.13.1)	μ Bondapak C18	300x4	ACN-MeOH-phosphate buffer(pH 6.0) (16:5:79)	7
Colchicine,demecolcine,N-des- acetylcolchicine,3-demethylcol- chicine,2-demethylcolchicine, 1-demethylcolchicine,ethylcol- chicinate,ethylisocolchicinate, N-methylcolchicineamide	Separation colchine deriva- tives	μ Bondapak C18 μ Bondapak Phenyl Lichrosorb RP8 10 μ m Lichrosorb RP18 10 μ m	300x4 150x4 250 100x4.1	ACN-MeOH-0.022M phosphate buffer (pH 6)(16:5:79)	8
Colchicine,colchicineamide,N-me- thyl- and N,N-dimethylcolchi- cineamide	Determination in microbial cultures	Ultrasphere ODS 5 μ m	250x4.6	ACN-MeOH-0.1M TrEA.HCl in 0.02M phos- phate buffer(pH 2.2)(200:85:715)	9

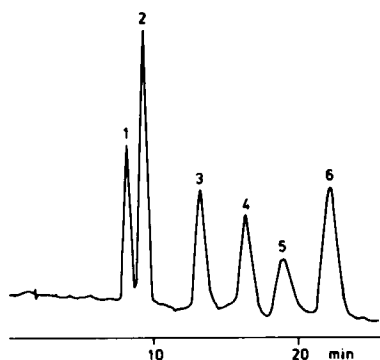


Fig. 13.1. Separation of colchicine and some derivatives⁷

Column μ Bondapak C18 (300x4 mm ID), mobile phase acetonitrile - methanol - phosphate buffer (0.038 M potassium dihydrogen phosphate, 0.005 M dipotassium hydrogen phosphate, pH 6.0) (16:5:79), flow rate 2 ml/min, detection UV 350 nm. Peaks: 1, 3-demethylcolchicine; 2, 2-demethylcolchicine; 3, N-desacetylcolchicine; 4, 1-demethylcolchicine; 5, N-methyl-N-desacetylcolchicine; 6, colchicine.

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Chapter 14

IMIDAZOLE ALKALOIDS

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14.1. HPLC SYSTEMS

Pilocarpine is widely used in ophtalmology in eye-drops. However, in solution it may undergo epimerization to isopilocarpine, and even hydrolysis to pilocarpic acid. In both cases loss of pharmacological activity is the result. Therefore, the analysis of ophtalmic solutions for pilocarpine and its decomposition products have been the subject of several investigations.

The first methods described for the analysis of pilocarpine used a cation-exchange resin in combination with a basic buffer^{1,2,3}. DeGraw et al.¹ and Urbanyi et al.³ used a TRIS buffer, but it was found to degrade column performance². A sodium phosphate buffer - tried instead of the TRIS buffer - caused problems as to the reproducibility of the separation².

Khalil⁴ used an octadecyl and a cyanopropylsilane column in series in order to analyze pilocarpine in the presence of preservatives in ophtalmic preparations. Tetrahydrofuran - borate buffer (pH 9.2)(3:7) was used as mobile phase.

Noordam et al.^{5,9} reported the separation of pilocarpine, and the decomposition products mentioned above, on an octadecyl column with a mobile phase consisting of a mixture of water and methanol (97:3) containing 5% potassium hydrogen phosphate (pH 2.5)(Fig.14.1). It was found that increasing the salt concentration and lowering the pH lead to improved selectivity and peak shape.

Kennedy and McNamara¹¹ found that replacing the octadecyl type of column in the method of Noordam et al.^{5,9} with a phenyl type of stationary phase reduced the analysis time, whilst improving peak shapes and resolution of pilocarpine and its degradation products. As mobile phase, 5% potassium dihydrogen phosphate (pH 2.5) in water was found to be the most suitable.

Kneckze⁶ applied ion-pair HPLC to analyze ophtalmic solutions containing pilocarpine, physostigmine and rubreserine (see Chapter 8, Table 8.7, Fig.8.7).

To improve the sensitivity of the pilocarpine analysis - enabling determinations in biological fluids - Mitra et al.⁷ developed a derivatization technique by means of which pilocarpine was quaternarized with the aid of *p*-nitrobenzylbromide (0.25 mg/ml - 24 hrs at 40°C). The quaternary derivatives were analyzed on an octadecyl column using 0.001 M sodium octanesulfonate in methanol - water (4:1) as mobile phase. the derivatization technique described was also applicable to other amines.

Straight-phase HPLC was used by Dunn et al.¹⁰, whereby the alkaloids were separated on silica gel with hexane - 2% ammonia in propanol (7:3). To avoid interference of UV absorbing nitrate, samples were passed through an ion-exchange column prior to HPLC.

Bundgaard and Hansen¹² separated pilocarpine and its degradation products on a silica gel column using methanol - 2 M phosphoric acid - water (3:5:92) containing 3% sodium sulfate as mobile phase (Fig.14.2). The poor separation of pilocarpine and isopilocarpine at a column temperature of 20-25°C was improved by increasing the temperature to 40°C.

Detection of pilocarpine is most sensitive at its UV maximum of 215 nm. However, detection at 220 nm improves the stability of the baseline, whereas only a minor decrease - 5% - of the peak height of pilocarpine is observed as compared with 215 nm¹⁰. The detection limit at 215 nm is about 0.04 µg, the detection limit obtained for the refractive index method is about 6 µg⁹.

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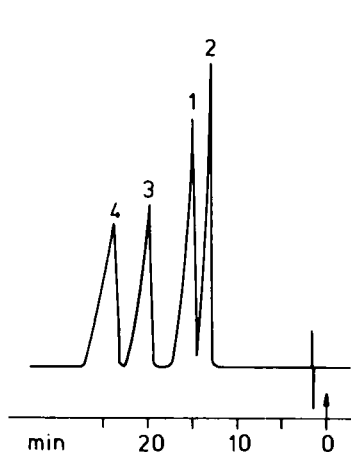


Fig. 14.1. Separation of pilocarpine and degradation products⁵
Column Lichrosorb RP18 10 µm (300x4 mm ID), mobile phase 5% potassium dihydrogen phosphate in water - methanol (97:3)(pH 2.5), flow rate 1.5 ml/min, detection with differential refractometer. Peaks: 1, pilocarpine; 2, isopilocarpine; 3, pilocarpic acid; 4, isopilocarpic acid.

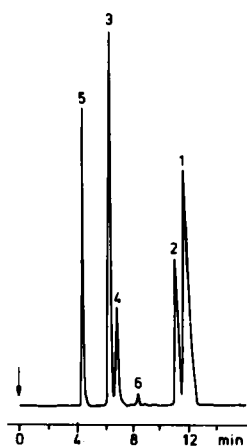


Fig. 14.2. Separation of pilocarpine and degradation products¹²
Column Lichrosorb Si60 5 µm (250x4.6 mm ID), mobile phase methanol - 2 M phosphoric acid - water (3:5:92) containing 3% of anhydrous sodium sulfate, flow rate 1.2 ml/min, detection UV 214 nm. Peaks: 1, pilocarpine; 2, isopilocarpine; 3, pilocarpic acid; 4, isopilocarpic acid; 5, nitrate; 6, unknown decomposition product.

TABLE 14.1

HPLC ANALYSIS PILOCARPINE AND RELATED ALKALOIDS

ALKALOIDS*	OTHER COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
Pil, isopil		Analysis synthesized pil	Aminex A-7	100x6	0.2M Tris buffer(pH 9.2) in 5% isoprOH	1
Pil, isopil, pilac		Analysis ophtalmic solutions	Aminex-7 7-11 μ m	65x5.5	0.1M Na-phosphate buffer(pH 9.0) in 5% isoprOH	2
Pil, isopil		Analysis in pharmaceuticals	Aminex-7 7-11 μ m	100x6	0.2M Tris buffer-isoprOH(95:5)(pH 9)	3
Pil, benzalkonium	Hydroxypropyl-methylcellulose	Analysis in pharmaceuticals	μ Bondapak C18 and μ Bondapak CN in series	300x4 300x4	THF-borate buffer(pH 9.2)(3:7)	4
Pil, isopil, pilac, isopilac		Analysis in pharmaceuticals (Fig.14.1)	Lichrosorb RP18 10 μ m or Nucleosil C18	300x4 150x4	5% KH ₂ PO ₄ in H ₂ O-MeOH(97:3)(pH 2.5)	5,9
Pil, physostigmine, rubreserine	Salicylate, phenethyl alcohol, methylparaben	Analysis in pharmaceuticals (Table 7.7, Fig.7.7)	μ Bondapak C18	300x3.9	MeOH-0.005M aq. heptanesulfonic acid (pH 3.6)(2:3)	6
Pil, isopil		Analysis in biological fluids by derivatization pil	μ Bondapak C18	300x3.9	MeOH-H ₂ O(4:1) containing 0.001M Na-oc-tanesulfonate	7
Pil, isopil, pilac, isopilac		Analysis in pharmaceuticals	Lichrosorb RP18 10 μ m	250x4.6	5% KH ₂ PO ₄ in H ₂ O-MeOH(97:3)(pH 2.5)	8,13
Pil, isopil		Analysis in pharmaceuticals	Si60 Hibar 5 μ m	250x4.6	Hexane-2% NH ₄ OH in isoprOH(7:3)	10
Pil, isopil, pilac, isopilac		Analysis in pharmaceuticals	μ Bondapak Phenyl	300x3.9	5% KH ₂ PO ₄ in H ₂ O(pH 2.5)	11
Pil, isopil, pilac, isopilac		Analysis pil degradation products in basic aqueous solutions (Fig.14.2)	Lichrosorb Si60 5 μ m	250x4.6	MeOH-2M H ₃ PO ₄ -H ₂ O(3:5:92) containing 3% Na ₂ SO ₄	12

* pil=pilocarpine, isopil=isopilocarpine, pilac=pilocarpic acid, isopilac=isopilocarpic acid

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Chapter 15

QUATERNARY AMMONIUM COMPOUNDS

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15.1. HPLC systems

The analysis of quaternary nitrogen compounds by HPLC requires chromatographic systems, such as ion-pair and reversed-phase chromatography, which are able to separate highly polar compounds. Reviews on ion-pair HPLC have been given^{10,16}. Both straight-phase (adsorption and/or partition) and reversed-phase ion-pair chromatography have been used.

Eksborg and Schill¹ separated a series of alkylamines on a cellulose column loaded with a 0.06 M aqueous picrate solution, of pH 11.2, using as mobile phase: chloroform - 1-pentanol (19:1). Addition of the alcohol to the mobile phase was necessary to reduce tailing. Celite and porous silica gel were found to be less suitable as solid support for aqueous picrate solutions. At the low pH necessary to ionize tertiary, secondary and primary amines, picrate eluted from the column (pH<6). Better results could be obtained for the amines mentioned with β -naphthalenesulfonate as counter-ion². A cellulose or celite support could be impregnated with 0.1 M aqueous solution of this counter-ion (pH 2.4), and by using chloroform - 1-pentanol, saturated with the stationary phase (as mobile phase) reproducible results were obtained.

Eksborg³ reported the separation of alkylammonium compounds as ion-pairs, using chloride as counter-ion. The support, diatomaceous earth, was coated with 0.05 M hydrochloric acid and chloroform - 1-pentanol (19:1) was used as mobile phase. To permit sensitive UV detection of the alkylamines, a second indicating column was coupled with the separating column. On the indicating column the chloride ions were exchanged by strong UV absorbing β -naphthalenesulfonate counter-ions. Diatomaceous earth was used in the indicating column as solid support and a solution of 0.1 M β -naphthalenesulfonate in 0.1 M hydrochloric acid was used as stationary phase.

Optimization of the system has been described by Crommen^{5,12}. He used microparticulate low surface silica gel as solid support for a solution of β -naphthalenesulfonate in a phosphate buffer of pH 2.3. The solid support was impregnated with the stationary phase by pumping the stationary phase through the solid support, when packed in the column. After the column is completely filled with the stationary phase, the mobile phase - saturated with the stationary phase - is pumped through the column until a clear eluent comes from the column. Injection of the stationary phase until droplets of it were eluted from the column was also used in order to impregnate the solid support with the stationary phase. Amino acids, dipeptides and alkylamines could be separated and sensitively detected by this method.

For quaternary alkylamines, a 0.1 M naphtalesulfonate solution in choline citrate buffer (pH 3.8) has been used as stationary phase on low surface silica gel, and with chloroform - 1-pentanol (9:1) saturated with the stationary phase as mobile phase¹⁸. Because a change of the stationary phase is time consuming, the retention of the compounds to be analyzed is usually regulated by changing the ratio of the solvent used in the mobile phase (Fig.15.1).

Hackzell and Schill²¹ later found that an alkanediol modified silica gel was more suitable as support for the aqueous stationary phase. Best results were obtained with rather high loads of stationary phase (Fig.15.2).

Greving et al.^{14,19} analyzed some basic drugs and quaternary ammonium compounds by means of ion-pair chromatography in the straight-phase adsorption mode. Bromide or perchlorate were used as counter-ions in connection with microparticulate silica gel columns. Chloride and iodide were less suitable as counter-ions, because they caused, respectively, corrosion of the equipment or a too strong UV absorption background of the mobile phase. Methanol was used as mobile phase, containing 0.01-0.1 M of the counter-ion.

Homologous series of quaternary alkylamines have been separated on porous microspherical polystyrene-divinylbenzene gel by means of methanol - containing perchloric, sulfuric or hydrochloric acid as mobile phase (Figs.15.3 and 15.4)⁴. The retention behaviour of the alkylamines could be explained in terms of partition chromatography.

Reversed-phase liquid-liquid ion-pair chromatography has been used to separate some tertiary and quaternary ammonium drugs¹⁵. Microparticulate porous silica gel was used as solid support. It was impregnated with lipophilic alcohols (1-pentanol, butanol). The aqueous mobile phase contained buffered salt solutions and 1% of the lipophilic alcohol. Retention of ionic samples could be increased by ions of the opposite charge (ion-pair effect) and decreased by ions of the same charge (competition effect). Various cationic and anionic components were discussed. Perchlorate, nitrate and 10-camphorsulfonate were used as pairing-ion (see also Chapter 4, Fig.4.7).

Johansson et al.⁸ separated some organic ammonium compounds by means of ion-pair chromatography using microparticulate chemically bonded octadecyl columns, that were impregnated with 1-pentanol or dichloromethane as stationary phase. The aqueous mobile phase - a pH 2.2 phosphate buffer saturated with the stationary phase - contained the counter-ion. Tailing could be reduced by adding a long chain tertiary or quaternary ammonium compound to the mobile phase. Dihydrogen phosphate, bromide, cyclohexylsulfamate, dicyclohexylsulfamate and octylsulfate were used as pairing-ions. Antidepressiva, neuroleptic amines and related quaternary ammonium compounds were analyzed⁹.

Ellipticine and related quaternary alkaloids have been analyzed on an octadecyl column using heptane- or pentanesulfonate as pairing-ion (see Chapter 8, Tables 8.5 and 8.6).

Some quaternary acetylcholine esterase inhibitors were analyzed by the Ruyter et al.¹⁷ on an octyl column using heptanesulfonic acid containing mobile phase (Fig.15.5). Best column performance was observed at lower pH. Addition of tetramethylammonium to the mobile phase reduced tailing on some of the tested reversed-phase column materials. Tetramethylammonium could also be used to regulate the retention of the compounds analyzed. The quaternary compounds were isolated from biological material by means of ion-pair extraction.

Van der Maeden et al.⁶ determined tubocurarine in curare samples. The influence of cations and pH on the separation of the alkaloids on an octadecyl column were studied. Optimum pH was found to be 4. Better peak performance and increased resolution was obtained by using tetramethylammonium as cation in the mobile phase, as compared with mobile phases containing potassium or ammonium ions. Optimum separation was obtained with gradient elution (see Chapter 6, Fig.6.3).

Berberine has been analyzed on an octadecyl column as ion-pair with dodecylsulfate^{20,22,26}. Reversed-phase separation on an octadecyl column has been used for the analysis of berberine, using a mobile phase of acetonitrile - phosphate buffer (pH 5.2)(3:2)⁷.

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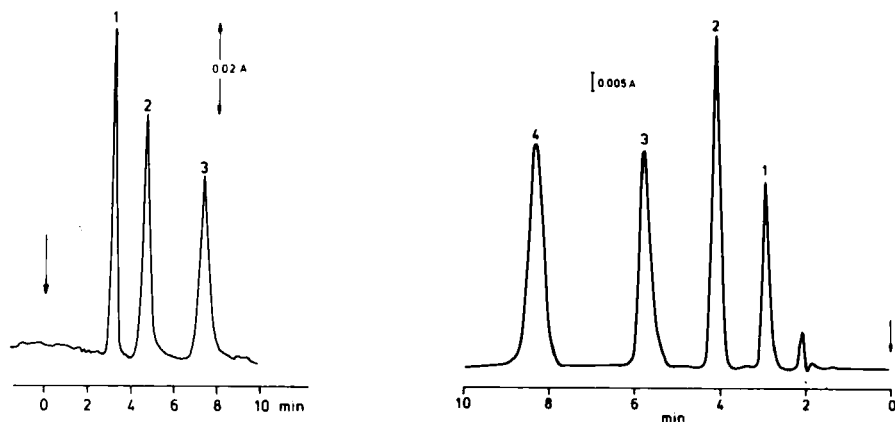


Fig. 15.1. Separation of some quaternary ammonium ions¹⁸

Column Lichrosorb Si500 10 μm , coated with 0.1 M naphthalene-2-sulfonate in 0.1 M choline citrate buffer (pH 3.8) (250x4 mm ID), mobile phase chloroform - 1-pentanol (9:1) saturated with the stationary phase, flow rate 1.0 ml/min, detection UV 254 nm. Peaks: 1, tripropylbutylammonium bromide; 2, tetrapropylammonium bromide; 3, tripropylmethylammonium iodide.

Fig. 15.2. Separation of some quaternary ammonium compounds²¹

Column Lichrosorb Diol 10 μm (150x3.2 mm ID) loaded with 0.1 M naphthalene-2-sulfonate in 0.1 M aqueous phosphate buffer (pH 2.1) by subsequently pumping 30 ml of phosphate buffer (pH 2.1) and 50 ml of the stationary phase through the column, followed by the mobile phase until no more droplets could be observed in the eluate (ca. 20 ml). Finally the column was recycled with 500 ml of mobile phase. Mobile phase chloroform - n-propanol (9:1) and chloroform - n-propanol (9:1) saturated with the stationary phase mixed in a ratio (1:9), flow rate 0.5 ml/min, detection UV 254 nm. Peaks 1, tetrabutylammonium; 2, tributylmethylammonium; 3, tetrapropylammonium; 4, tripropylmethylammonium. (reproduced with permission from ref. 21, by the courtesy of Acta Pharmaceutica Suecica).

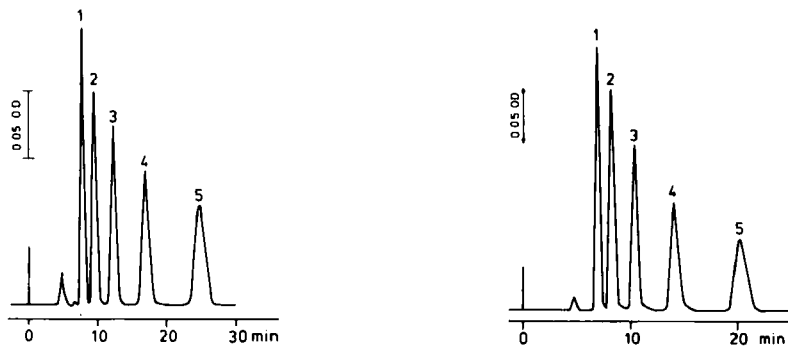


Fig. 15.3. Separation of alkylbenzyltrimethylammonium chlorides⁴

Column Hitachi Gel 3011 (500x4 mm ID), mobile phase 0.5 M perchloric acid in methanol, flow rate 1.1 ml/min, column temperature 30 $^{\circ}$ C, detection UV 220 nm. Peaks: 1, decyl- ; 2, dodecyl- ; 3, tetradecyl- ; 4, hexadecyl- ; 5, octadecyl-benzyltrimethylammonium chloride.

Fig. 15.4. Separation of alkylpyridinium chlorides⁴

Column Hitachi Gel 3011 (500x4 mm ID), mobile phase 0.5 M perchloric acid in methanol, flow rate 1.1 ml/min, column temperature 30 $^{\circ}$ C, detection UV 260 nm. Peaks: 1, decyl- ; 2, dodecyl- ; 3, tetradecyl- ; 4, hexadecyl- ; 5, octadecylpyridinium chloride.

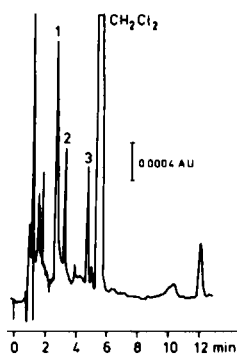


Fig. 15.5. Analysis of neostigmine in serum sample¹⁷

Column 5 μ m Ultrasphere Octyl (150x4.6 mm ID), mobile phase 0.01 M heptanesulfonate, 0.01 M sodium dihydrogen phosphate and 0.0025 M tetramethylammonium chloride in acetonitrile - water (1:4) (pH 3.0), flow rate 2 ml/min, detection UV 214 nm. Peaks: 1, interference; 2, edrophonium (internal standard); 3, neostigmine.

TABLE 15.1

HPLC ANALYSIS QUATERNARY AMMONIUM COMPOUNDS

COMPOUNDS	AIMS	STATIONARY PHASE	COLUMN DIM. LxID(mm)	MOBILE PHASE	REF.
Quat. alkylamines	Separation by ion-pair partition chromatography	Cellulose 30-65 μm loaded with 25% 0.06 M picrate solution (pH 11.2)	300x2.7	CHCl_3 - n -AmOH(19:1) sat. with the stationary phase	1
Alkylamines	Ion-pair chromatography of organic compounds	Cellulose 30-65 μm or Celite 37-74 or 15-37 μm , loaded with 0.06M picrate solution(pH 11.2) or 0.1M naphthalenesulfonate solution(pH 2.4)	300x2.7	CHCl_3 - n -AmOH(19:1) sat. with the stationary phase	2
Alkylamines	Ion-pair chromatography separation followed by transformation into UV-absorbing ion-pair	Dia-Chrom 37-44 μm loaded with 25% 0.05M HCl	300	CHCl_3 - n -AmOH(19:1) sat. with the stationary phase	3
Alkylbenzyltrimethylammonium-,alkylpyridinium halides	Separation (Fig.15.3, 15.4)	Hitachi gel 3011 10-15 μm	500x4	0.5M HClO_4 in MeOH	4
Alkylamines, amino acids, dipeptides	Ion-pair chromatography with highly UV-absorbing counter ions	Lichrospher Si100 10 μm loaded with 0.01M naphthalenesulfonate in pH 2.2 phosphate buffer	150x4.5	CHCl_3 - n -AmOH(95:5),(9:1) sat. with the stationary phase	5,12
Tubocurarine, chondrocurarine, curarine, isochondrodendrine, curine	Analysis curare	μ Bondapak C18	300x4	0.025M tetramethylammonium phosphate in A. MeOH- H_2O (1:3)(pH 4) B. MeOH- H_2O (9:11)(pH 4) in 30 min linear gradient A+B(9:1) to (3:17)	6
Berberine	Analysis in <i>Coptis</i> species	μ Bondapak C18	300x4	ACN-phosphate buffer(pH 5.2)(3:2)	7
Phenylethylamines, neuroleptic amines, quaternary ammonium compounds	Separation by reversed-phase ion-pair HPLC	Lichrosorb RP8 10 μm dynamically coated with n -AmOH	150x3.2	0.028M Dimethyloctylamine in 0.1M pH 2.2 phosphate buffer, sat. with n -AmOH 0.043M NaBr, 0.028M dimethyloctylamine in 0.1M pH 2.0 phosphate buffer, sat. with n -AmOH	8,9
Tubocurarine, isochondrodendrine, curine	Analysis in plasma	μ Bondapak C18	300x4	ACN- H_2O (18:82) containing 0.2M HClO_4 (pH 5.4)	11

Ellipticine and quaternary derivatives	Separation (Table 8.5, 8.6)	μ Bondapak C18	300x4	MeOH-0.005M heptanesulfonic acid, 0.032M AcOH(7:3), (3:1) MeOH-0.005M pentanesulfonic acid, 0.032M AcOH(7:3) MeOH-0.02M NH_4OAc (3:1)	13
Quaternary ammonium derivatives and basic drugs	Separation by ion-pair adsorption chromatography	Lichrosorb Si60, Si100 or Lichrospher 5 μm	300 or 150x4	Various conc. NaBr or NaClO_4 in MeOH	14, 19
Atropine, scopolamine, methylatropine, various drugs	Separation by reversed-phase ion-pair HPLC (Fig. 4.7)	Lichrospher Si100 10 μm	200x4	0.1M Na-phosphate buffer (pH 2.2) + 1.9% AmOH	15
Pyridostigmine, neostigmine, edrophonium and their 3-hydroxy metabolites	Analysis in biological fluids	Ultrasphere Octyl 5 μm	150x4.6	0.01M Heptanesulfonate, 0.01M NaH_2PO_4 and 0.0025M tetramethylammonium chloride in $\text{ACN-H}_2\text{O}$ (1:4), (17:83) (pH 3)	17
Quaternary alkylamines	Separation (Fig. 15.1)	Lichrospher Si500 10 μm loaded with 0.1M naphthalenesulfonate in pH 3.8 choline citrate buffer	250x4	CHCl_3 -n-AmOH(9:1) sat. with the stationary phase	18
Berberine, acrinol	Analysis pharmaceuticals	Zorbax ODS	250	0.005M Dodecylsulfate in $\text{ACN-H}_2\text{O}$ (95:5)	20
Quaternary alkylamines	Determination by using UV-absorbing counter-ions (Fig. 15.2)	Lichrosorb DIOL 10 μm loaded with 0.1M naphthalenesulfonate in aq. phosphate buffer (pH 2.1)	150x3.2	CHCl_3 -prOH(9:1) sat. with the stationary phase to various degrees	21
Berberine, palmatine	Histochemical chromatography	Lichrosorb RP8	not given	ACN-THF -0.1N tartaric acid-Na dodecylsulfate(20:20:59.5:0.5)	22
Methylhomatropine, codeine, morphine, noscapine, papaverine, thebaine	Analysis pharmaceuticals	Nucleosil 5C8	120x4.6	ACN -0.01M phosphate buffer (pH 5.0) (2:3)	23
Alcuronium, tubocurarine	Analysis in biological fluids	μ Bondapak C18	300x6.4	MeOH- H_2O (4:1) containing 0.25% AcOH and 0.005M Na-dodecylsulfate	24
Tubocurarine	Analysis in plasma	Radial-Pak C18	100x5	MeOH-(TrEA(10g/l)-pentanesulfonic acid (1ml)- H_3PO_4 (2ml)- H_2O ad 1l)(2:3)	25
Berberine, palmatine, coptisine	Analysis in plant material	TSK gel LS-410 5 μm	150x4	ACN-MeOH -0.1N tartaric acid-Na dodecylsulfate(40:10:49.5:0.5)	26
Ellipticine, 9-hydroxy-ellipticine, 11-demethylellipticine	Analysis in biological material	μ Bondapak C18	300x4	ACN -0.01M NaH_2PO_4 (36:64), (25:75) (pH 3.5)	27

Appendix

Description of some stationary phases, which have been reported in this book. The list is not a complete list of available stationary phases, for such a summary is referred to refs 1 and 2.

Ion-exchange packings					
Name	Manufacturer/Supplier	particle size μm	materials	ion-exchange capacity meq/g	
Aminex A7	Bio-Rad	7-11	polystyrene-divinyl benzene resin (SO ₃ ⁻)	5	
Nucleosil SA	Macherey Nagel & Co	5,10	silica gel (SO ₃ ⁻)	1	
Partisil SCX	Whatman	10	silica gel (SO ₃ ⁻)	1	
Vydac TP401 SCX	Macherey Nagel & Co	10	silica gel (SO ₃ ⁻)	1	
Zipax SAX	DuPont	25-37	laurylmethacrylate polymer coated pelliculars (NR ₃ ⁺)		
Zipax SCX	DuPont	25-37	SO ₃ ⁻ , pellicular		
Zipax MAX	DuPont	25-37	laurylmethacrylate polymer coated pelliculars		
Reversed-phase packings					
Name	Manufacturer/Supplier	particle size μm	bonded functional group	particle shape	% C loading
μBondapak C18	Waters Associates	10	octadecylsilyl	irr*	10%
Corasil C18	Waters Associates	37-50	octadecylsilyl	pell	
Hypersil ODS	Shandon	6	octadecylsilyl	spher	9%,endcapped
Lichrosorb RP18	E.Merck	5,10	octadecylsilyl	irr	22%
Micropak MCH	Varian	5,10	octadecylsilyl	irr	12%

Nucleosil C18	Macherey Nagel & Co	5,7.5,10	octadecylsilyl	spher	15-16%
ODS Sil-X-I	Perkin Elmer	13	octadecylsilyl	irr	
ODS Sil-X-II	Perkin Elmer	35+15	octadecylsilyl	pell	
Partisil ODS-1	Whatman	5,10	octadecylsilyl	irr	5%
Partisil ODS-2	Whatman	10	octadecylsilyl	irr	15%
Partisil ODS-3	Whatman	10	octadecylsilyl	irr	10%,endcapped
Spherisorb ODS	Phase Separation Ltd	5,10	octadecylsilyl	spher	7%,endcapped
Ultrasphere ODS	Altex	3,5	octadecylsilyl	spher	12%,endcapped
Zipax ODS	Dupont	25-37	octadecylsilyl	pell	
Zorbax ODS	Dupont	6,8	octadecylsilyl	spher	15%
Chromagabond C8	ES Industries	5,10	octylsilyl	irr	15%
Lichrosorb RP8	E.Merck	5,10	octylsilyl	irr	13-14%
Nucleosil C8	Macherey Nagel & Co	5,7.5,10	octylsilyl	spher	10-11%
Ultrasphere Octyl	Altex	5	octylsilyl	spher	6.5%,endcapped
Spherisorb C6	Phase Separation Ltd	5	hexylsilyl	spher	endcapped
Chromagabond C6H11	ES Industries	10	cyclohexylsilyl	irr	10%
Hypersil SAS	Shandon	3,5,10	short alkylsilyl	spher	3%
Lichrosorb RP2	E.Merck	5,10	dimethylsilyl	irr	
Corasil Phenyl	Waters Associates	37-50	diphenylsilyl	pell	
μBondapak Phenyl	Waters Associates	10	diphenylsilyl	irr	10%

Polar chemically bonded packings

Name	Manufacturer/Supplier	particle size μm	bonded functional group	particle shape	% C loading
μBondapak CN	Waters Associates	10	cyano	irr	9%
Lichrosorb CN	E.Merck	5,10	cyano	irr	
Nucleosil CN	Macherey Nagel & Co	5,10	cyano	spher	
Spherisorb CN	Phase Separation Ltd	5	cyano	spher	
μBondapak NH ₂	Waters Associates	10	alkylamino	irr	9%

Micropak NH ₂	Varian	10	alkylamino	irr
Nucleosil NH ₂	Macherey Nagel & Co	5,10	alkylamino	spher
Lichrosorb Diol	E.Merck	10	hydroxy	irr
μBondapak Carbohydrate	Waters Associates	10		irr
Durapak OPN	Waters Associates	37-75	oxydipropionitrile	irr
Zipax ETH	Dupont	25-37	ether groups	pell
Hamilton PRP1	Hamilton	10-15	polystyrenediviny- benzene resin(CH ₂ OH functional groups)	
Hitachi gel 3011	Hitachi	10-15	polystyrenediviny- benzene resin(CH ₂ OH functional groups)	

Normal-phase packings

Name	Manufacturer/Supplier	particle size μm	pore size nm	particle shape	surface area m ² /g	pore volume ml/g
Hypersil	Shandon	6	10	spher	200	
Lichrosorb Si 60	E.Merck	5,7,10,30	6	irr	475	0.76
Lichrosorb Si 100	E.Merck	5,7,10,30	10	irr	278	1.02
Lichrospher Si 100	E.Merck	5,10,20	12	spher	256	1.20
Lichrospher Si 500	E.Merck	10	50	spher	45	0.88
Micropak Si(identical with Lichrosorb Si 60)	Varian	5,10	6	irr	475	0.76
Nucleosil 50	Macherey Nagel & Co	5,7.5,10	5	spher	500	0.80
Nucleosil 100	Macherey Nagel & Co	5,7.5,10	10	spher	300	1.00
Partisil	Whatman	5,10,20	4-5	irr	400	0.70
Porasil A	Waters Associates	37-75	10	spher	350-500	1.05
Porasil T	Waters Associates	15-25,25-37	15	spher	300	
μPorasil	Waters Associates	10	10	irr	400	1.00
RSil	Alltech	5,10	6	irr	550	
Sil-X-1	Perkin Elmer	13	10	irr	400	

Spherisorb SW	Phase Separation Ltd	3,5,10	8	spher	220	0.6
Spherosil XOA 800	Rhône-Progil	5,10	3	spher	830	0.4-0.6
Spherosil XOA 600	Rhône-Progil	5,10,20	6	spher	600	0.7-1
Spherosil XOA 400 (identical with Porasil A)	Rhône-Progil	10	10	spher	350-500	1.05
Spherosil XOA 200	Rhône-Progil	10	15	spher	125-250	0.9
Vydac TP Adsorbent	Macherey Nagel & Co	10	33	irr	100	0.6
Zorbax Sil	DuPont	6	7.5	spher	300	

* irr=irregular, pell=pellicular, spher=spherical

References: 1. K.K.Unger, Porous Silica, J.Chromatogr.Library Vol.16, Elsevier, Amsterdam, 1979.
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INDEXES

How to find your way

The various subjects discussed in the chapters are presented in the Subject Indexes, as well as the botanical names of plants mentioned in the text. Solvent systems, detection methods and various chromatographic techniques (ion-exchange, reversed-phase, ion-pair, straight-phase) are not included in the Subject Index. These subjects are dealt with separately for each group of alkaloids and may be found in the list of contents of each chapter.

All compounds with a pertinent reference to retention times or detection methods either in the text, figures or tables are listed in the compound index. For the various groups of alkaloids, tables summarizing all the available literature are presented at the end of each chapter. Compounds listed in these tables are not included in the Compound Index. Hence to find all data relating to a particular compound, it may be necessary to consult both the Compound Index and the appropriate tables.

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